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Further Investigations into Immunization of Cattle against Rinderpest.

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INTRODUCTION.

In a subsequent paper by Mitchell and Mansfeld, on work carried out at a temporary field station at Mbosi, it will be pointed out that certain results were obscured and certain conclusions might be invalidated by a doubt as to the full susceptibility to rinderpest of some of the available cattle used for experimental purposes. Whether this resistance to infection was due to natural variation in the susceptibility of the local animals, or to immunity developed as a result of contact with the disease in an enzootic area, could not be determined. Since additional and confirmatory evidence on various aspects of immunity and immunization was urgently required for the formulation of a scheme to be applied to the rapid elimination of any outbreak of rinderpest, which might occur among the fully susceptible cattle of the Southern African states, it was decided to continue the work in an area where the results would not be open to question. Eventually, the Government of Northern Rhodesia agreed to permit the work to be carried out in the northern portion of their territory and Col. Gore-Browne, a member of the Government, generously offered to provide a site, facilities for the erection of the requisite kraals, and accommodation for staff on his estates at Shiwa Ngandu.

The experimental animals used were drawn from several sources.

1. Col. Gore-Browne again generously offered to loan part of his herd of cattle for the work. These animals referred to in the text as Shiwa cattle, were grade stock (South Devon-Shorthorn-Zebu crosses).

2. The Northern Rhodesia Government supplied animals from their herds at District Commissioner's headquarters at Mpika some 65 miles south of Shiwa (referred to as Mpika boma cattle) and from Chungu about 150 miles north east of Shiwa (referred to as 'hunga cattle).

These three herds of cattle were isolated groups in local tsetse fly free areas surrounded at some distance by fly belts. No rinderpest had been encountered in the area for at least 30 years. The natives in the vicinity of Shiwa owned some goats but no cattle, so that the risk of spreading infection was reduced to a minimum.

PROCEDURE.

The experimental work was carried out at Shiwa and Mpika in two main stages; firstly from September to November 1940 (two months), and secondly, in June and July 1942 (one month). The test inoculations at Chunga were carried out during November, 1941.

While the experimental work was in progress the animals were kraaled at night, and were temperatured at sunrise before being turned out to graze in appropriate groups. Some modification of this general procedure had to be made in the case of the animals in the transmission tests but it must be emphasized that a temperature record was kept of each individual whether in an actual experiment at the time or not.

When the first stage of the work was discontinued in November, 1940, all the animals were rebranded for future identification, the Shiwa herd being kept in reserve by Col. Gore-Browne, the remainder being controlled by the Veterinary Department of Northern Rhodesia. On resuming the work in June, 1942, it was found that a large number of the animals had died of intercurrent disease, or for other reasons were no longer available. This is the explanation for the unfortunate gaps in the protocols and is an indication of the difficulties under which this work was carried out.

To minimize the risk of spreading infection only goat virus was used in the tests. This Kabete type goat virus (referred to as K.G.V.) was supplied by the Veterinary Research Laboratory, Kabete, Kenya. It was sent to Mpika by air mail, where it was placed on ice in a Thermos flask, transported by car and stored in a refrigerator. Unless specifically stated to the contrary, K.G.V. refers to a fresh emulsion of this stored desiccated spleen virus.

EXPERIMENTAL.

1. *To repeat the investigation previously carried out at Mbosi on the possible transmission of K.G.V. from reacting to susceptible animals.*

(a) *Close contact.*—On 17/10/40 a group of 23 head of cattle was placed in a small boma (kraal). Ten, to comprise the infected group, received an injection of K.G.V. All, with one exception, No. 18, reacted severely, five showing severe clinical symptoms of diarrhoea with nasal and lachrymal discharges. The non-reactor, No. 18, which resisted infection on two subsequent occasions, is discussed later. Arrangements were made for this group of infected and in-contact animals to be fed and watered by hand. In addition to a plentiful supply of food, salt was fed *ad. lib.* in an open trough. They were not allowed out to graze but were retained continuously in the kraal for a period of 3 weeks by which time the reactors had fully recovered and were allowed out to grass. The in-contacts then received an immunity test of K.G.V. the dose being 2 c.c.; all reacted severely, 12 showing marked clinical symptoms.

Result.—Infection was not transmitted to 13 susceptible cattle during close contact with 9 reactors throughout the entire period of the rinderpest reaction to K.G.V.

(b) *Open grazing.*—On 17/10/40 75 head of cattle on open grazing received an injection of K.G.V. A total of 50 susceptible animals were maintained in the infected area and reacting herd as in-contacts from October 20th to November 8th, 1940, i.e. for a period of 19 days.

Results.—(a) During the period of exposure 8 of the in-contacts showed a fluctuating rather than a definite rise of temperature. The highest recorded temperature was only 102.8° but this was several degrees higher than the average of the remaining animals.

Blood was collected from these 8 animals for subinoculation into goats. As the number of available rinderpest susceptible goats was limited, 4 goats each received the pooled blood of two cattle. All the goats showed a slight febrile reaction (maximum temperature 105.2°) between the 6th and 13th day. The reactions in the goats were all delayed 3-5 days beyond the normal incubation period of rinderpest in goats and no clinical symptoms developed. On testing with virus all the susceptible cattle, including the 8 mentioned above, reacted at the normal period showing in some cases marked clinical symptoms.

These doubtful reactions in the 8 cattle were not considered to be due to rinderpest infection as the cattle were still susceptible twelve days after the first rise of temperature. If these doubtful reactions were caused by rinderpest virus, some, if not all, would have failed to react to the K.G.V. immunity test inoculation.

It was concluded that some other virus, transmissible to goats, had been picked up from the cattle. Had time permitted, these goats would have been tested with K.G.V. for immunity to rinderpest but the experimental work was then closing down and this could not be done. (When the immunity tests were conducted at Chunga in June, 1941, a second rise of temperature followed the normal K.G.Virus reaction in some of the animals and blood from these animals caused a slight rise in temperature, similar to that observed at Shiwa, in two subinoculated goats. Lack of time once again prevented further investigation of the virus).

(b) One in-contact animal (No. 103) commenced a thermal reaction on the 14th day of contact, the temperature rising to a maximum of 104.6° on the 16th day and slowly returning to normal on the 23rd day. Smear examination showed the presence of a heavy infection of *Theileria mutans* and the development of anaemia. Blood was collected on the 4th day of the reaction and 10 c.c. was subinoculated into a goat (No. 26). A febrile reaction commenced on the 3rd day rising to 106° on the 6th day and returning to normal on the 13th day. Blood from goat 26 was subinoculated into a second goat, 28, on the 4th day of the febrile reaction. Again a febrile reaction commenced on the 3rd day rising to 105.4° on the 6th day and returning to normal on the 8th day. In none of the animals could any clinical symptoms of rinderpest be detected.

Conclusion.—It is not possible to draw any definite conclusion from the experiment. It is quite apparent that nine of the in-contact cattle became infected with a febrile condition transmissible to goats. In the case of 8 animals this febrile condition was evidently not due to the transmission of K.G.V. as the animals proved susceptible to an immunity test dose twelve days after the first rise of temperature. In the case of the ninth animal, (No. 103), there is a possibility that the K.G.V. was transmitted. From the nature of the reaction and the absence of well marked clinical symptoms, however, the opinion is held that the K.G.V. was not transmitted.

(c) *Drenching and inoculation.* (i) *Urine.*—On 25/10/40 urine was collected from 5 reactors to K.G.V. immediately after slaughter on the 5th day of the reaction. Two head of cattle were each drenched with 20 c.c. of the fresh urine immediately after collection and a further two each received 10 c.c. subcutaneously.

Result.—None of the animals showed any reaction and, on testing their immunity 15 days later, all reacted.

(ii) *Faeces.*—Fresh faeces were collected from two reactors showing profuse blood-stained diarrhoea on the 8th day after infection with K.G.V. Immediately after collection 2 cattle were drenched with 20 c.c. of the pooled material. A further sample was passed through a Berkefield candle, the process of filtration taking several hours at a high room temperature. Two animals each received 10 c.c. of the filtrate subcutaneously.

Result.—One animal which was drenched with faeces developed a febrile reaction with clinical symptoms indicative of rinderpest, and failed to react to an immunity test given 15 days later. The other 3 animals failed to react and on testing their immunity 15 days later were found to be susceptible.

Conclusion.—From this series of experiments it would appear to be safe to conclude that transmission of attenuated goat virus under conditions of close contact or open grazing, if it does occur, is exceedingly rare. However the possibility that such transmission may occur by the ingestion of food contaminated with faecal material from reacting animals is proved by the single positive transmission obtained by drenching infected faeces.

2. To determine the duration of immunity produced by vaccination with formolized spleen vaccine.

(a) During November all the cattle in the estate herd at Shiwa received a single injection of 10 c.c. of Mbosi formol-glycerine vaccine as additional security against the possibility of disseminating infection. A group of 6 heifers from this herd was selected in July, 1941, i.e., 8 months after immunization and they were given an immunity test in the form of the subcutaneous injection of 5 c.c. of fresh goat blood virus collected from donors reacting to the standard injection of K.G.V.

Result.—All 6 heifers showed marked febrile reactions indistinguishable from those produced in unvaccinated controls.

Conclusion.—After an interval of 8 months the immunity conferred by a single injection of formol-glycerine vaccine had disappeared completely.

(b) A group of 9 cows and 10 calves was purchased for test from Mbesuma ranch 200 miles north-east of Shiwa (Rumsey's cattle) where triple vaccination with freshly prepared formol-saline vaccine had been carried out 8 months previously. The calves, whose history is somewhat obscure, had not been vaccinated but may have developed some transient immunity as a result of the ingestion of antibody contained in colostrum. The cows and calves were given an immunity test of K.G.V.

Result.—Marked febrile reactions were produced in 4 cows and 7 calves, the cows showing clinical symptoms of rinderpest. None died.

Conclusion.—After an interval of 8 months the immunity produced by triple vaccination with formol-saline vaccine had diminished considerably, the decrease apparently being correlated with an idiosyncrasy of individual animals.

(c) A group of 64 Chunga cattle was triple vaccinated with freshly prepared formol-saline vaccine in February, 1940. On October 2nd, 1940, these cattle received a reinforcing injection of 10 c.c. of Mbosi formol-glycerine vaccine. In June, 1941, i.e., 17 months after the original triple vaccination and 9 months after the second single injection a standard immunity test of K.G.V. was given.

Result:—

No reaction	45
Doubtful febrile reaction	8
Febrile reaction	11
Clinical reaction	0

Conclusion.—Triple vaccination followed by a single reinforcing injection of vaccine 9 months later is followed by an immunity which persists for 9 months. This conclusion is justified by the finding that 45 of the 64 animals were solidly immune and that a considerable degree of immunity had persisted in the 11 reactors since the febrile reactions were exceedingly mild. This result is of prime importance and affords a striking contrast to the decrease in immunity observed 8 months after triple vaccination alone [cf. previous experiment (b)].

To determine whether the immunity had been sufficient to block the reaction to the K.G.V. completely and thus prevent the development of a durable active immunity, the entire group of 64 cattle were given a second immunity test of K.G.V. in November, 1941, i.e. 5 months later.

Result.—All the previous reactors and doubtful reactors proved to be immune. Of the 45 non-reactors 43 were immune and two showed very mild febrile reactions.

Conclusion.—A durable immunity had been produced in the animals.

3. Vaccine-Virus Immunization Tests.

Using the transient immunity produced by formolized spleen-virus vaccine to control the reaction produced by a fully virulent or partially attenuated virus in an endeavour to develop a durable active immunity, is a standard procedure requiring no elaboration. Since the application of the method to immunization against rinderpest has received little attention, a detailed investigation was started at Shiwa. Unfortunately a true appreciation of the results is negated by the fact, as stated previously, that a number of the animals were not available for the subsequent immunity tests.

For the experiments the vaccine used was formol-glycerine vaccine prepared at Mbosi on 12/6/40, stored at Iringa for use in Tanganyika and is referred to in the text as Iringa vaccine. A further consignment of the same batch of vaccine had been sent from Iringa for use in the Sumbawanga area. Some of this vaccine was obtained from Sumbawanga for use at Shiwa and is referred to in the text as Sumbawanga vaccine. This vaccine had been stored at Sumbawanga in a grass hut for four months.

IMMUNIZATION OF CATTLE AGAINST RINDERPEST.

The virus used was the fresh emulsion of desiccated Kabete Goat virus, previously referred to. Dose of vaccine 10 c.c. Dose of virus 2 c.c. subcutaneously.

In the tables the interval stated refers to the time that elapsed between the injection of vaccine and the virus. These injections were commenced in October, 1940. The immunity tests, which consisted of 5 c.c. of freshly drawn blood obtained from goats reacting to K.G.V. given subcutaneously, were carried out in June, 1942.

TABLE 1.
Vaccine-Virus Immunization.

	Interval in Days.	No. of Cattle.	FEBRILE REACTION.*			Clini- cal Re- actions.	IMMUNITY TEST.			
							Non-Reactors.		Reactors.	
			Neg.	Mild.	Marked.		Pos.	Neg.	Pos.	Neg.
Shiwa grade cattle	6 7 11 15 19 19 (a) 22	10 15 15 15 11 15	— 4 10 11 14 3 11	6 7 4 2 1 5 3	4 4 1 2 — 3 1	4 3 — — — 6 —	— — 3 6 7 5	— — 2 0 1 1	— — — — 1 (d) —	— 4 — — 2 —
Mpika Zebra type cattle	7 (b) 7 (c)	10 15	6 9	4 4	— 2	1 5	0 0	5 10	1 (d) 0	3 3
Controls.....	—	2	—	—	2	2	—	—	0	2

* In this and other similar tables reactions are divided into two classes:—

- (1) Febrile reactions (Negative, Mild and Marked) as indicated by the record of daily temperatures.
 - (2) Clinical reactions, where a diagnosis could be made from the symptoms. It is apparent, therefore, that the clinical reactors are included amongst the febrile reactors.
- (a) The test on this 19 day interval group was carried out at a different time during the rains, when the cattle were showing signs of diarrhoea and severe reactions.
- (b) Sumbawanga vaccine.
- (c) Iringa vaccine. *
- (d) Very mild and possibly doubtful reactions.

Results.—Consideration of the reactions indicated in Table I shows that, as the interval between the injection of vaccine and of virus increased, so the severity of the resultant reactions decreased and the number of non-reactors increased. An exception is the second batch of 11 animals in the 19 day interval group. These animals were treated after the rains had begun and it was a constant observation that as soon as the new grass began to appear a large percentage of cattle showed diarrhoea and all lost condition. Any rinderpest reactions which developed during this period were markedly accentuated.

As regards the immunity produced it is indeed regrettable that all survivors of the experiment were not available for test, since it is of paramount importance to ascertain whether a non-reactor to an attenuated virus develops a permanent immunity or not. Since the immunity tests were carried out after an interval of 20 months, i.e. at a time when practically all residual immunity conferred by the vaccine should have disappeared (see above) any immunity must have resulted from a reaction to K.G.V. It is worthy of note, therefore, that in the case of the Mpika boma cattle, where the interval between vaccine and goat virus was 7 days, all the non-reactors developed a solid immunity. In the case of the Shiwa grade cattle none of the 7 day group non-reactors were tested but 3 out of 5 of the 11 day interval group were resistant. Non-reactors from the longer interval series were not immune in the great majority of instances (16 out of 18).

Conclusion.—As the interval between a single injection of vaccine and K.G.V. increases so the severity of the reactions as well as the percentage of reactors decrease. When the interval is 7 days it can be expected that both non-reactors and reactors, i.e., all animals, will develop a solid persistent active immunity. When the interval is longer than 7 days the immunity produced by the vaccine is sufficient not merely to control the reaction produced by the attenuated virus but to block it completely; development of an active immunity will be prevented and the transient immunity produced by the vaccine after rising to a peak in an undetermined interval will decline until it is no longer effective after 8 months.

4. *The efficacy of formal-glycerine spleen prepared from Kabete goat virus.*

It has been shown that there is an almost negligible danger of Kabete goat virus spreading from reacting to susceptible cattle whereas there is a very real danger of the spread of ordinary cattle virus. In any extensive immunization campaign involving the preparation of large quantities of formalized spleen-vaccine it is frequently necessary to establish the vaccine production plant in close proximity to the source of susceptible cattle, i.e. outside the immune zone or belt. This necessitates the adoption of vigorous precautions to prevent further spread of the very disease whose elimination is aimed at, and there always exists an unpleasant feeling of doubt that a new focus of infection is being established. It is an accepted phenomenon that the vaccine produced from a different genus of animal from that which is to be immunized is less effective than vaccine produced from the same species. Consequently it was considered inadvisable to investigate the value for cattle of spleen-vaccine prepared from goats but some information on the antigenic value of cattle spleen-vaccine prepared from K.G.V. would be of interest.

The K.G.V. vaccine used in the following experiments was prepared at Shiwa from the pooled spleens of 10 oxen killed at the height of the reaction on the 5th day after infection with K.G.V. The method of preparation of vaccine was identical with that used at Mbosi, the percentage of formalin being 0.25 per cent. The vaccine was prepared in two batches, the first batch being used on 21 Mpika boma cattle, the second batch on 15 Shiwa cattle. The reactions and results are shown in Table 2.

IMMUNIZATION OF CATTLE AGAINST RINDERPEST.

TABLE 2.

K.G.V. Vaccine-Virus Immunization.

Cattle.	No.	Interval in Days.*	FEBRILE REACTION.			Clinical Re- action.	IMMUNITY TEST.			
							Non-Reactors.		Reactors.	
			Neg.	Mild.	Marked.		Pos.	Neg.	Pos.	Neg.
Mpika.	} 21	7	4	6	11	13	0	2	1 (b)	15
Boma..		8	0	6	9	7 (a)	—	—	—	—
Shiwa..	15									

* Interval refers to time between injections of vaccine and virus.

(a) 1 died; rinderpest.

(b) Very mild or doubtful reaction.

Results.—The reactions produced should be considered in conjunction with those reported with ordinary cattle spleen-vaccine in Table 1. The reactions were far more severe, in fact one of the Shiwa grade animals actually died of rinderpest. On the other hand only two Mpika boma non-reactors whose immunity was tested 20 months later were both solidly immune.

Conclusion.—It may be concluded that formol-glycerine spleen-vaccine prepared from cattle reacting to attenuated Kabete goat virus has a lower antigenic value for cattle than a similar product where virulent cattle virus is used as the source of infection.

5. Duration of Immunity.

All animals which showed a febrile reaction to Kabete goat virus were subsequently found to be immune. Under the experimental conditions the limit of interval between infection and immunity test was 22 months.

DISCUSSION.

Although every effort was made to bring a series of carefully planned experiments on fully susceptible grade cattle in a rinderpest free area to their logical conclusion it was found that some of the difficulties under which the work was carried out could not be overcome. This is exemplified by the fact that the requisite immunity tests on many of the most important animals in various groups could not be carried out simply because these animals had either succumbed to intercurrent disease or for other reasons were no longer available when finally required. Nevertheless the results and the justifiable conclusions drawn indicate the necessity for careful investigation into immunity production in a rinderpest free area on grade cattle whose full susceptibility to rinderpest is not open to question. It must be noted, however, that even though these two requirements appeared to be fulfilled at Shiwa yet a single animal, No. 18, was encountered

which was completely refractory to infection. It is certain that this animal had not been in contact with the disease previously, yet on three occasions it failed to react to fully active virus. No explanation of this phenomenon can be given.

The first conclusion that may be drawn from the whole series of experiments is that Kabete goat virus is not sufficiently attenuated for use on the average type of animal to be found in the Southern African states. Although only a single death was recorded, the reactions produced in the control animals in the various groups were severe and were frequently accompanied by well-defined clinical symptoms of rinderpest. This point should be borne in mind when any extensive campaign is planned in the future.

Insufficient work was carried out to determine the best method of controlling these severe reactions; for instance, the simultaneous use of hyper-immune serum and K.G. Virus was not investigated. However, it should be borne in mind that at least in the Union of South Africa the initiation of a campaign of control would probably be a matter of extreme urgency and great speed, and consequently, a supply of serum would not be available in adequate amount. For this reason the results produced by the use of formolized spleen-vaccine are important.

Vaccine produced from cattle spleens using Kabete goat virus as the infecting agent for the donors possesses inferior antigenic properties and cannot be recommended. However, the combination of what may be termed standard formolized vaccine with attenuated goat virus appears to hold out considerable promise. Since the term 'standard vaccine' has been used with set purpose it is necessary at this stage to emphasize that no technique has yet been evolved to determine the antigenic value of different batches of formolized cattle spleen-vaccine. Vaccine is prepared by a particular technique from a sufficiently large number of spleens to justify the hope that the antigenicity of different batches will vary only within very narrow limits, but this cannot be controlled by any rapid *in vitro* method, so that a comparison of the results of different experiments, particularly by different workers, may not be justified.

From the results of the experiments under consideration it appears that the rapid production of immunity by formolized spleen-vaccine may be used to control the reaction produced by goat virus, and a factor of extreme importance is the interval between the injection of vaccine and virus. If the interval is less than 7 days sufficient immunity will not have been produced and a high percentage of severe reactions will occur subsequently. If the interval is greater than 11 days the immunity may be sufficient to block out the reaction to goat virus completely and a permanent immunity will not be produced. An interval of 7 days appears to be the optimum for adequate control of the attenuated virus and the development of the highest percentage of animals with a lasting active immunity.

As far as the dissemination of the disease is concerned the use of Kabete virus appears to be reasonably safe. No case of transmission under conditions of close contact was recorded and only one doubtful case on open grazing. The record of a febrile condition transmissible from cattle to goats indicates the great care that must be taken in evaluating the results of work of this nature. No dogmatic denial of the possibility of transmitting goat virus

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from reacting to susceptible cattle can be made since a positive transmission was obtained by drenching infected faeces. However, under natural conditions the danger of transmission appears to be remote.

The decrease in immunity produced by spleen-vaccine after an interval of 6 months is in accordance with current opinion but the rapid stimulation of a more durable immunity by a single reinforcing injection of vaccine 9 months after triple vaccination is a finding which merits further investigation.

SUMMARY.

1. Kabete goat virus was not transmitted from reacting to susceptible cattle under conditions of close contact.
2. A single doubtful transmission was recorded under conditions of open grazing.
3. A febrile condition of unknown aetiology transmissible from cattle to goats was encountered.
4. Urine from reacting animals was non-infective, but faeces in one out of two cases was infective by drenching.
5. Immunity produced by a single injection of formol-glycerine spleen-vaccine had completely disappeared after 8 months.
6. Immunity produced by triple vaccination with formol-saline vaccine had diminished considerably after 8 months.
7. Triple vaccination followed by a single injection of formol-glycerine spleen vaccine 9 months later produced an immunity which persisted for at least 20 months.
8. The rapid production of immunity induced by a single injection of formol-glycerine spleen-vaccine could be used to control the reaction to K.G.V. An interval of 7 days between vaccine and virus appeared to be the optimum.
9. Spleen-vaccine prepared from cattle reacting to K.G.V. has an inferior antigenic potency.
10. The reaction produced by K.G.V. in grade cattle (British breeds of cattle \times Zebu) are severe but usually non-fatal. A durable immunity follows the reaction.

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On the Etiology of Epizootic or Infectious Equine Abortion.

By M. W. HENNING, Section of Bacteriology, Onderstepoort.

IN a previous article Henning, Keppel and Flight (1943) described an outbreak of infectious abortion in mares in the Cape Province. Further evidence in regard to this outbreak is given, another outbreak is described and the results of a number of transmission experiments are recorded.

Since Kilborne (1893) and Smith (1893) incriminated an organism of the hog-cholera group as the causal agent of infectious abortion of equines several workers in different parts of the world have described this disease. Good and Corbett (1913) and Good and Smith (1914) isolated an organism of the "*enteritidis cholerae-suis group*" from the after-birth and foetal organs of aborted foetuses and called it *Bacillus abortivo-equinus*. By intravenous and intraperitoneal inoculations of saline suspensions of cultures of this bacterium they induced pregnant mares to abort in about ten days' time and isolated the organism from the foetal membranes and foetal organs. The disease was also studied in great detail by de Jong (1912), van Heelsbergen (1914), Murray (1919) and others. These authors all found that the majority of abortions occurred from 7-9 months; they also succeeded in producing abortion in pregnant mares by means of intravenous or intraperitoneal inoculation of cultures of paratyphoid organisms which they had previously isolated from the after-birth and foetal organs, but failed to induce premature birth when the cultures were given by the mouth or per vaginam. Later the bacterium associated with equine abortion was called *Salmonella abortus-equi*.

Abortus-equi was generally accepted as the only important etiological agent of infectious abortion in equines until Dimock and Edwards (1936), Dimock (1940), and Dimock, Edwards and Bruner (1942), incriminated a filterable virus as the cause of abortion in some outbreaks of abortion studied by them. But they still regarded *abortus-equi* as a very common causal agent. In agreement with other workers they found that abortion seldom recurs in the same mare a second time, and that a diagnosis of *abortus-equi* abortion can be made by recovering the salmonella from the after-birth and foetal organs, and also by means of agglutination tests. They did not regard the stallion as an important factor in the dissemination of the disease. Like Ostertag (1901) they attributed some forms of infectious abortion in mares to a streptococcus which was thought to enter the body by way of the genital tract and not the mouth.

Dimock, Edwards and Bruner studied several outbreaks of infectious abortion in mares in which cultivable organism could not be incriminated as the cause and in which the serum of affected mares consistently gave negative agglutination tests. They succeeded in inducing abortion in both pregnant guinea-pigs and pregnant mares by means of unfiltered and filtered suspensions of foetal organs. Guinea-pigs that received Seitz filtrates gave birth either to premature or dead foetuses from 14 to 29 days after injection. Of the ten pregnant mares that received filtered foetal material three aborted on the 18th, 23rd and 24th days respectively after the inoculation. The remainder gave birth to normal foals. The aborted foetuses presented pathological changes that were regarded as characteristic of the disease. Another mare placed in an infected stable and fed on infected foetal membranes aborted after 24 days and the foetus showed lesions resembling those of the naturally occurring disease. All

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the foetuses dropped by the experimental mares were found to be bacteria-free. The authors therefore, concluded that a filterable virus should be regarded as the cause of these abortions.

In the outbreaks studied there was an abortion incidence of more than 70 per cent. and more than 90 per cent. of the foetuses were found to be free from cultivable bacteria. The mare apparently suffers no inconvenience and the foetal membranes are not retained. Involution of the genital organs takes place as readily as after a normal parturition and the mare is usually ready to be bred about a week later. The pregnancy that now follows will usually run a normal course and a healthy foetus will be delivered at full time.

The most characteristic pathological changes presented are small multiple, greyish-white areas of degeneration in the liver, accumulation of serous, usually blood-stained, fluids in the peritoneal and pleural cavities, small haemorrhages on the epi- and endocardium, swelling of the spleen and sometimes congestion of the lymph glands of the colon. The amnion may be oedematous. The majority of abortions occur between the 8th and 10th months. In late pregnancies the foals may be born alive but then they usually die within 36 hours. The infection is highly contagious and may spread very rapidly but the stallion is not considered to play a rôle in the dissemination of the disease nor is the recovered mare considered to act as a carrier. The disease does not recur in the same stud during two successive years but may recur after the lapse of 3 to 5 years.

These workers found acidophilic intranuclear inclusion bodies in sections of the liver, lung and epithelium of the respiratory tract of the foetus. The basophilic chromatin of the nucleus is retracted from the centre to lie on the inner margin of the nuclear membrane, leaving a clear space around the inclusion bodies. For diagnostic purposes a histological examination of the liver and lungs is recommended. It is stated that the intranuclear inclusion bodies will be readily found in the epithelium of the bronchioles, bronchi and alveoli, and also in the hepatic cells near the periphery of the necrotic nodules. Propagation of the virus in developing chick embryos was not possible.

Later Hupbauer (1938), Miessner (1938), and Manninger and Csontos (1941) studied a similar condition in Europe. Miessner (1938) and his collaborator, Harms, investigated a highly contagious epizootic of abortion in an exceedingly well-managed stud in Germany during 1936 and had to exclude *abortus-equi* and *streptococci* as causal agents. Infection was thought to occur at pasture where the whole number of mares were running together. The aborting mares apparently suffered no ill effects and the after-births were normally expelled. A number of the foals that were born alive died soon after birth. The lesions found resembled those described by Dimock and Edwards (1936), and a virus was also incriminated as the cause.

Hupbauer (1938) studied epizootic abortion of equines in Yugoslavia where abortions were comparatively common, but it was thought that losses could be prevented by the adoption of proper hygienic measures and inoculations with *abortus-equi* vaccine. In 1937 a particularly severe outbreak occurred about 14 days after the inoculation. The lesions found also resembled those described by Dimock and Edwards. By means of Seitz E.K. filtrates of foetal suspensions Hupbauer was able to induce abortion in both guinea-pigs and mares and concluded that a virus was the cause of these abortions.

The epizootic abortions investigated by Manninger and Csontos (1941), usually occurred between the 6th and 11th months of pregnancy. The lesions resembled those described in the above outbreaks and a virus was also incriminated. Successful transmission experiments were carried out in guinea-pigs and mares by means of both filtered and unfiltered suspensions of foetal organs. Characteristic lesions were revealed in the internal organs of the foals that were born alive as well as in the aborted foetuses.

Agglutination.—The evidence provided in the literature indicates that there is a marked divergence of opinion with regard to the agglutination titre of the sera of normal horses for *abortus-equi*. Thus Good and Corbett (1913), van Heeelsbergen (1914), de Jong (1912), and Verge (1939), regard the titre of a normal horse to vary from 1 : 200 to 1 : 300 as compared to a titre of 1 : 500 and over for an affected equine. Murray (1919), on the other hand, found that the titre of a normal horse seldom exceeded 1 : 40, while Saxer (1938), considered a titre of 1 : 100 as positive. More

recently Stitz and Görkel (1938), in making a serological survey of *abortus-equi* in a military camp, tested 237,687 horses of which 766, including 497 mares, had an agglutination titre of 1 : 800 and over; but they noticed that the titre fluctuated considerably during a short time. Moreover, these authors isolated *abortus-equi* from 128 equines that had died from a variety of diseases, in which this organism could not be associated with the clinical picture or the lesions presented. They also isolated *abortus-equi* from 2 out of 408 samples of faeces, from 8 out of 398 samples of urine, and from 19 out of 67 samples of uterine discharge. Some of the carriers were geldings and some gave a negative agglutination reaction to *abortus-equi*. They, therefore, regarded this organism as a secondary invader of horses, except when found in the genital tract.

In the agglutination carried out by these authors it is not stated whether "H", "O", or mixed "O" and "H" antigens were used.

In order to determine the agglutination titre of the serum of normal animals for *abortus-equi* several routine samples of serum from equines, cattle, and sheep submitted to Onderstepoort for various purposes were tested serologically. Both "O" and "H" antigens were employed in these tests.

Of 128 samples of horse serum mostly from mares, submitted from different parts of the country, 13 gave "O" agglutination of 1 : 40, 33 of 1 : 20, 41 of 1 : 10 and the remainder (41) failed to react at these dilutions. The "H" agglutination was either lower or very little higher than the "O". But as Henning and Haig (1940) pointed out, for the detection of carriers, "O" agglutination alone can be relied upon, so that not much attention was paid to the "H" agglutination. In addition, 242 samples of bovine serums were tested; of these, three gave an "O" reaction of 1 : 40, 28 of 1 : 20 and the rest (211) had a titre of 1 : 10 or less. From these results it seems reasonable to assume that equine sera with an "O" agglutination titre of 1 : 40 should be regarded as negative. If the reaction is over 1 : 80 the serum is probably positive for *abortus-equi*.

Several agglutination tests were carried out at regular intervals with the sera of the equines involved in the Smal outbreak described by Henning, Keppel, and Flight (1943). The results of these tests are given in Table 1.

The sera of two mares taken some time after abortion were submitted to Onderstepoort for examination. The one sample was haemolysed while the other gave a strongly positive reaction for *abortus-equi*, and a diagnosis of *abortus-equi* infection was made. Arrangements were then made for the examination of sera from all the equines in the stud. The results of the agglutination tests obtained with these sera (Table 1) were not quite clear as the majority of the mares that were known to have aborted from one to three months previously gave negative agglutination reactions while others reacted positively (compare Tables 2, 4 and 6). Sera submitted from two pregnant mares, Nos. 34 and 35, were also negative. Later both these mares aborted and the serological reactions obtained with their sera were regarded as significant.

Mare No. 35 aborted on 26th September, 1942, i.e., one day after her serum had a negative reaction; but when she was bled nearly 3 weeks later (11th October) her serum was positive. Mare No. 34, which aborted about a month later, was still negative on the day of abortion but had become positive within 19 days. The sera of both mares 34 and 35 remained positive

for 2-3 months. *Abortus-equi* was readily recovered from all the foetal organs and the after-births of both these mares; the foetal organs of no other mare were available for examination.

In order to ascertain whether a virus as described by Dimock and Edwards (1936) was present in these *abortus-equi* infected foetal organs, preliminary transmission experiments were carried out with 8 pregnant guinea-pigs and one pregnant mare, using collodion filtrates of foetal organ suspensions as inoculum. The guinea-pigs were inoculated either by the subcutaneous or the intraperitoneal routes, receiving from 2 to 4 c.c. each; the mare was inoculated intravenously with approximately 20 c.c. of the filtrate.

Three of the guinea-pigs that aborted before the 7th day were discarded from the experiment, but the remaining five either aborted or gave birth to weaklings after an incubation period which varied from 9 to 19 days. Nearly all the guinea-pigs that were born alive died after a day or two. The mare delivered a live but very weak foal on the 22nd day; the foal refused to drink and died on the third day. Apart from the presence of abnormal amounts of blood-stained fluids in the pericardial, pleural, and peritoneal cavities no pathological changes were observed. The necrotic nodules described by Dimock and Edwards (1936), could not be seen and a bacteriological examination of the foetal organs was negative. No further material was available and the experiment was interrupted until August, 1944, when a very severe outbreak of infectious equine abortion occurred in the Animal Embarkation Depot at Pinetown, where a number of equines brought from different parts of the country were collected and kept in small camps prior to export to India.

THE PINETOWN OUTBREAK.

Foetal organs preserved in 50 per cent. glycerine were submitted by Capt. B. M. McIntosh to Onderstepoort for examination. In all, organs from eight horse and four donkey foetuses were submitted. Of these, four horse foetuses and all four donkey foetuses yielded *abortus-equi* on culture; from four of the horse foetuses no *abortus-equi* could be obtained. Only horse and donkey foetal organs that were found to be positive for *abortus-equi* were used for transmission experiments. An exception was made in the case of the foetal organs of mare 683 (*vide infra*). Sera obtained from all the affected mares were tested at the time of abortion and again about 2 weeks later, and in most cases again at varying intervals subsequently (Table 2). It will be noticed that, with the exception of mares T.1082 and T.604, the sera of all the mares taken at the time of abortion were negative. The sera of all those whose foetuses yielded *abortus-equi* on culture became positive from 1-2 weeks afterwards, remained positive for some time and then the titre gradually dropped. With the exception of mare T.604 the sera of all the mares whose foetuses were negative for *abortus-equi* remained negative afterwards. The serum of this mare (T. 604), however, was found to be positive on the day of abortion given.

Transmission Experiments.—In the Pinetown outbreak it was found that approximately 90 per cent. of the pregnant horse mares kept at the animal Embarkation Depot aborted. A few gave birth to live foals, but these foals developed joint-ill and had to be destroyed. Large numbers of equines were confined to small camps so that conditions were very favourable for the dissemination of the infection, once it was introduced. A number

TABLE 1.
Outbreak of Equine Abortion H. J. Smal (1942).
Serological Tests with Abortus-Equi, "H" and "O," Antigens.

No. of Animal.	Date of Abortion.	Date of Bleeding.	O.	H.	Date.	O.	H.	Date.	O.	H.	Date.	O.	H.	Date.	O.	H.
1.	11/6/42	25/9/42	20	20	14/11/42	20	20	V2/8/43	40	20	V11/8/43	200	12,800	20/8/43	400	50,000
2.	July/August	25/9/42	100	160	14/11/42	40	80	—	—	—	—	—	—	—	—	—
3.	July/August	25/9/42	20	20	14/11/42	20	80	V2/8/43	40	40	V11/8/43	200	1,200	20/8/43	800	50,000
4.	6/9/42	25/9/42	80	320	14/11/42	160	160	—	—	—	—	—	—	—	—	—
5.	June 1942	25/9/42	10	40	14/11/42	10	40	V19/9/43	40	80	V20/9/43	6400	54,000	E27/5/43	1600	3,200
6.	July/August	25/9/42	10	40	14/11/42	20	80	V2/8/43	20	20	V11/8/43	200	1,000	20/8/43	400	12,800
7.	July/August	25/9/42	20	10	14/11/42	40	160	V2/8/43	200	100	V11/8/43	100	6,100	20/8/43	1600	6,400
8.	July/August	25/9/42	20	40	14/11/42	20	40	V2/8/43	10	20	V11/8/43	100	6,100	20/8/43	200	12,800
9.	July/August	25/9/42	20	40	14/11/42	20	40	V2/8/43	40	20	V11/8/43	200	3,200	20/8/43	400	50,000
10.	July/August	25/9/42	20	40	14/11/42	20	80	V2/8/43	10	40	V11/8/43	100	3,200	20/8/43	1600	6,400
11.	July/August	25/9/42	320	640	14/11/42	40	320	V2/8/43	80	80	V11/8/43	100	800	20/8/43	800	50,000
12.	July/August	25/9/42	10	40	14/11/42	10	10	2/8/43	20	20	F31/8/43	20	40	10/9/43	20	—
13.	July/August	25/9/42	80	160	14/11/42	80	320	V2/8/43	160	100	V11/8/43	100	50,000	20/8/43	1000	100,000
14.	July/August	25/9/42	100	160	14/11/42	80	160	V2/8/43	20	20	V11/8/43	200	3,200	20/8/43	1600	50,000
15.	July/August	25/9/42	100	160	14/11/42	80	160	21/2/43	80	0	—	—	—	—	—	—
16.	July/August	25/9/42	100	160	14/11/42	80	160	2/8/43	80	0	—	—	—	—	—	—
20.	15 August	25/9/42	80	160	14/11/42	40	80	2/8/43	80	20	24/9/43	80	40	16/10/43	40	20
22.	July/August	25/9/42	80	160	14/11/42	20	80	V19/4/43	80	80	V20/4/43	200	6,100	8/5/43	200	50,000
30.	August	25/9/42	10	80	14/11/42	20	80	14/11/42	160	640	V2/8/43	40	20	V11/8/43	200	3,200
34.	27/10/42	25/9/42	10	80	27/10/42	10	40	11/10/42	320	640	2/3/43	80	320	2/8/43	160	40
35.	26/9/42	25/9/42	20	80	25/9/42	20	40	—	—	—	—	—	—	—	—	—
Stallion 44	Swollen Testis	25/9/42	80	640	14/11/42	320	1280	—	—	—	—	—	—	—	—	—
Stallion 45	—	25/9/42	20	160	19/11/42	40	160	—	—	—	—	—	—	—	—	—

F = Fouled.

V = Inoculated with abortus-equi Vaccine.

* Reaction on 2.3.43 was O = 100, H = 800.

TABLE 2.
Agglutination Reactions of Sera Submitted from the Pinetown Outbreak.

No. of Animal.	Date of Abortion.	Foetal Organ Pos. or Neg. for ab. equi.	Date of Bleeding.	Abortus-equi.		Date of Bleeding.	Abortus-equi.		Abortus-equi.	
				O.	H.		O.	H.	O.	H.
T. 902.....	14/7/44	Pos.	9/8/44	320	640	11/10/44	40	80	—	—
T. 604.....	25/8/44	Neg.	25/8/44	320	320	11/10/44	40	20	—	—
T. 1899.....	6/9/44	Neg.	25/8/44	20	20	6/9/44	20	0	20	20
T. 1986.....	8/9/44	Pos.	8/9/44	20	20	15/9/44	640	320	160	80
T. 1082.....	11/9/44	Pos.	11/9/44	160	320	11/10/44	1280	2600	—	—
T. 828.....	14/9/44	Pos.	15/9/44	40	10	25/9/44	320	10,000	320	640
Donkey V. 1991.....	30/9/44	Pos.	30/9/44	20	40	15/10/44	1280	160	—	—
Donkey M. 138.....	3/10/44	Pos.	5/10/44	20	80	15/10/44	80	1,280	—	—
Donkey V. 4531.....	11/10/44	Pos.	11/10/44	80	320	20/10/44	320	320	—	—
T. 1983.....	11/10/44	Neg.	11/10/44	40	10	20/10/44	20	10	—	—
Donkey M. 55.....	11/10/44	Pos.	11/10/44	40	40	20/10/44	80	640	—	—
T. 1855.....	11/10/44	Neg.	11/10/44	40	40	20/10/44	40	10	—	—

TABLE 3.
Results of Transmission Experiments with *Latapi* Minced Infected Foetal
Organs or Live Cultures of Abortus-Equi Given by the Mouth.

No. of Animal.	Date of introduction into Experiment.	Nature of Experiment.	Result.	Result of Bact. Examination of Organs or Afterbirth.	Pathological Anatomy.
Horse 176.	17/8/44	Dosed with minced organs of foetus from Pinetown	Aborted on 2/9/44....	Foetal organs positive for <i>abortus-equi</i>	Sub-epi. and endocardial hæmorrhages. Hyperaemia of intestines.
Horse 21818	12/9/45	Dosed with minced foetal organs from Pinetown	Aborted on 1/10/44....	Foetal organs positive for <i>abortus-equi</i>	Hydrothorax, hydropericard., petechiae on surface of lungs and peritoneum. Hyperaemia of intestines.
Horse 682..	27/11/44	Dosed with minced foetal organs from Pinetown	Aborted on 2/1/45....	Foetal organs positive for <i>abortus-equi</i>	Sub-epi. and endocardial hæmorrhages, hyperaemia of lungs and liver, atelectasis, tumor splenis.
Donkey 691	27/11/44	Dosed with minced foetal organs of 20810	Aborted on 12/12/44....	Foetal organs positive for <i>abortus-equi</i>	Hydrothorax, hydropericard, ascites, sub-epi. and endocardial petechiae; petechiae on surface of spleen, tumor splenis, intense hyperaemia of intestine, degeneration of liver and kidneys.
Donkey 687	27/11/44	Dosed with infected joint-oil from foal of mare 22523	Foaled normally on 11/3/45	Afterbirth negative for <i>abortus-equi</i>	—
Donkey 704	30/12/44	Dosed with minced organs of foetus of 683 (<i>abortus-equi</i> free)	Foaled on 10/2/45; foal apparently normal	Afterbirth positive for <i>abortus-equi</i>	—
Donkey 705	30/10/44	Dosed with minced organs of foetus of 683 (<i>abortus-equi</i> free)	Aborted on 20/1/45....	Foetal organs positive for <i>abortus-equi</i>	Sub-epi. and endocardial hæmorrhages, hydropericard, tumor splenis, hyperaemia of intestines, stasis of liver.
Donkey 728	13/2/45	Dosed with <i>latapi</i> minced organs of 694 and 705	—	—	—
Donkey 693	5/12/44	Dosed with emulsion of 6 mason tube cultures in saline	Foaled on 28.12/44 Foal apparently normal	Afterbirth not examined..	—
Donkey 697	4/12/44	Dosed with emulsion of cultures from 6 mason tubes in saline	Foaled with difficulty on 19/1/45. Foal weak and died few hours later	Foetal organs negative for <i>abortus-equi</i>	Hyperaemia of liver and lungs.
Donkey 689	4/12/44	Dosed with emulsion of cultures from 6 mason tubes in saline	Foaled 22/3/45.....	Afterbirth negative for <i>abortus-equi</i>	—

of pregnant donkey mares, kept in the same camps as the horses, also picked up the infection, but, although several abortions occurred among the donkeys, the disease did not seem to affect them as seriously as horses, and a greater proportion of donkey foals were born alive. Moreover, the donkey foals that were born alive, remained apparently healthy and developed normally.

Pathological Anatomy.—According to Captain McIntosh the fetuses aborted during the Pinetown outbreak presented the following lesions to a varying degree:—Hydrothorax, hydropericardium, ascites, epicardial and endocardial haemorrhages, petechiae on surface of lungs and spleen, tumor splenis, degeneration of the liver, icterus, and oedema of the placenta.

Notwithstanding the apparently greater resistance of donkey mares to infectious equine abortion so few horse mares were available that a number of pregnant donkey mares had to be employed for some of the transmission experiments.

The material used for the transmission experiments included (1) latapi minced foetal organs, (2) filtrates of latapi minced organs, and (3) live suspensions of 24 hours old cultures of different strains of *abortus-equi*. The foetal organs used were first obtained from aborted fetuses at Pinetown, but later as abortions were experimentally produced at Onderstepoort, the organs of these fetuses were used.

A number of the donkey mares and one horse mare that were admitted into the transmission experiment on the assumption that they were pregnant had to be discharged subsequently as they turned out not to be in foal.

(A) *Transmission by Means of Latapi Minced Abortus-Equi Infected Foetal Organs (Tables 3 and 4).*

Exceptions mares 704 and 705.

1. *Horse mare 176* was dosed on 17th August, 1944, with minced *abortus-equi* infected foetal organs obtained from Pinetown. This mare aborted on 2nd September, 1944, i.e. 16 days later. All the foetal organs were positive for *abortus-equi* infection and the foetus showed the following lesions: Sub-epicardial and sub-endocardial haemorrhages, marked hyperaemia of intestinal mucosa. The serum of the mare was negative at time of abortion, but positive 13 days later.

2. *Horse mare 21818* was dosed on 12th September, 1944, with *abortus-equi* infected minced organs. It aborted on 1st of October, 1944, i.e. 19 days later. The foetal organs were positive for *abortus-equi* and the foetus presented the following lesions: Hydrothorax, hydropericardium, petechiae on surface of lungs and on parts of peritoneum, and hyperaemia of intestines. The serum of the mare was negative at the time of abortion, but positive 16 days later.

3. *Horse mare 682* was dosed with minced *abortus-equi* infected organs on 27th November, 1944. It aborted on 2nd January, 1945, i.e., 37 days after infection. All the foetal organs were positive for *abortus-equi* and the foetus presented the following lesions: Sub-epi- and endocardial haemorrhages, hyperaemia of lungs and liver, tumor splenis. The serum of this mare was negative at the time of abortion but positive two weeks later.

4. *Donkey mare 691* was dosed on 27th November, 1944, with organs of the foal of mare 20810 (see Tables 5 and 6) and aborted on 12th December, 1944. The foetus presented lesions of hydrothorax, hydropericardium, ascites, sub-epi- and endocardial haemorrhages, petechiae on surface of spleen, tumor splenis, severe hyperaemia of intestines, degeneration of liver and kidneys. The foetal organs were positive for *abortus-equi* and the serum of the mare, negative at the time of abortion, was suspicious or slightly positive two weeks later.

TABLE 4.

Serological Results of the Mares in Table 3 with Abortus-Equi "O" and "H".

No. of Animal.	Date of Abortion or Foaling.	Date of Test.	O. H.	Date of Test.	O. H.	Date of Test.	O. H.	Date of Test.	O. H.	Date of Test.	O. H.
Horse 176..	Aborted on 2/9/44....	2/9/44	10 10	15/9/44	320 320	23/9/44	80 320	2/11/44	40 20	—	—
Horse 21818	Aborted on 1/10/44...	12/9/44	0 10	3/10/44	20 40	17/10/44	640 5,000	19/2/45	40 160	—	—
Horse 682..	Aborted on 2/1/45....	27/11/44	10 10	2/1/45	10 40	16/1/45	320 80	8/2/45	640 80	—	—
Donkey 691	Aborted on 12/12/44..	27/11/44	40 40	12/2/45	40 40	27/2/45	80 40	8/2/45	40 40	—	—
Donkey 704	Foaled on 10/2/45....	30/12/44	0 10	10/2/45	0 40	26/2/45	20 40	10/3/45	10 20	—	—
Donkey 705	Aborted on 20/1/45...	30/12/44	0 20	20/1/45	0 20	3/2/45	160 80	8/2/45	320 160	10/3/45	40 80
Donkey 728	—	13/2/45	0 10	—	—	—	—	—	—	—	—
Donkey 693	Foaled on 28/12/45...	27/11/44	10 40	28/2/44	10 20	11/1/45	320 80	8/2/45	80 40	—	—
Donkey 697	Foaled on 19/1/45. Foal died 19/1/45	27/11/44	0 20	19/1/45	0 20	2/2/45	0 20	8/2/45	10 20	—	—
Donkey 689	Foaled on 22/3/45....	27/11/44	0 20	22/3/45	640 1,280	26/3/45	160 320	13/4/45	160 10	—	—
Donkey 687	Foaled on 11/3/45....	27/11/44	10 40	12/3/45	10 20	26/3/45	20 40	10/4/45	20 40	—	—

5. *Donkey mare* 704 was dosed on 30th December, 1944, with the minced organs of the foetus of horse mare 683 which were culturally negative for *abortus-equi*. She foaled on 10th February, 1945, and the foal appeared quite normal, but *abortus-equi* was isolated from the after-birth. The serum of this mare was negative at the time of abortion and remained negative afterwards.

6. *Donkey mare* 705 was dosed on 30th December, 1944, with the minced organs of the foetus of horse mare 683 which were culturally negative for *abortus-equi*. She aborted on 20th January, 1945, and the foetal organs were found to be positive for *abortus-equi*. Her serum was negative at the time of abortion but positive two weeks later. The foetus presented lesions of sub-epi- and endocardial haemorrhages, hydro-pericard, tumor splenis, hyperaemia of the intestine and stasis of liver.

Three donkey mares, viz., Nos. 693, 697 and 689 were each dosed on 4th December, 1944, by means of a stomach tube with the saline emulsion of 24 hours' growth of three strains of *abortus-equi* on 6 Mason tubes (Mason, 1933).

Mare No. 693 gave birth to a live healthy foal on 28th December, 1944. Her serum was negative on the day of abortion but positive 2 weeks later. The after-birth was not examined bacteriologically.

Mare No. 689 gave birth to a live foal on 22nd March, 1945, i.e., after 118 days. Her after-birth was negative for *abortus-equi*, but her serum was strongly positive on the day of abortion.

Mare No. 697 foaled with difficulty on 19th January, 1945, and the foal died a few hours after birth. Apart from hyperaemia of the lungs and liver no abnormal changes were revealed by the internal organs of this foetus. A bacteriological examination of the foetal organs was negative for *abortus-equi*; the serum of the mare was negative at the time of abortion and remained negative afterwards.

Mare 687 was dosed with infected joint fluid from the foal of horse mare 22523 on 27th November, 1944 (foal was suffering from *abortus-equi* infected joint-ill). She gave birth to an apparently healthy foal on 11th March, 1945. Her serum, negative on 12th March, i.e., one day after abortion, remained negative. The after-birth failed to yield *abortus-equi* on culture.

(B) *Transmission by Means of Filtrates (Tables 5 and 6).*

Abortus-equi infected foetal organs were minced in the latapi and approximately 10 gm. of the pulp was suspended in 30 c.c. isotonic saline. The suspension was spun for 3 hours at 3,000 r.p.m. in a Clay-Adams angle centrifuge. The supernatant fluid obtained was passed through an asbestos filter (Elford, 1938). The resultant filtrate was then filtered under two atmospheres of nitrogen, first through collodion membrane with the size of the pores 1,200 milli-micron, and finally through a membrane with the pores 810 milli-micron in size (Elford, 1938, Bauer and Hughes, 1935). The filtrations were carried out by Dr. A. Polson, of the Section of Virus Disease of this laboratory.

The filtrate was tested for sterility by inoculating approximately 2 c.c. either into tubes containing 15 c.c. broth each, or into 30 c.c. chopped meat broth. No growth was apparent during the first four or five days, but after about two weeks incubation a turbidity was noticed in some of the tubes. Sub-inoculation on to MacConkey, with seed material from the various tubes, yielded a growth in some cases but not in others. The growths obtained from the different tubes all yielded different types of colonies, but the colonies in the same culture appeared to be alike. The morphology of the organisms in the different cultures were also different, some being Gram-positive, coccus-like, while others were Gram-positive bacteria. No organisms could be found that could in any way be associated with a salmonella. The growth, obtained in these tubes were, therefore, regarded as due to contamination. Those tubes where no growth could be detected were considered to be bacteriologically sterile.

TABLE 5.
Results of Transmission Experiments with Filtrates of Infected Foetal Organs.

No. of Animal.	Date of entry into Experiment.	Nature of Experiment.	Result.	Result of Bacteriological Examination of Foetus or Foal.	Pathological Anatomy.
Horse 22523	17/8/44	Inoculated with collodion filtrate of foetal organ from Pinetown*	Foaled on 7/9/44. Foal apparently normal, but developed joint-ill later	Afterbirth negative for <i>abortus-equi</i>	
Horse 20810	5/9/44	Inoculated with collodion filtrate of foetal organ from Pinetown	Foaled on 25/10/44. Foal weak and died on 26/10/44	Organs of foal positive for <i>abortus-equi</i>	General icterus, extensive sub-endo- and sub-epicardial petechiae, Oedema of lungs, hyperaemia of stomach and intestines. Retention of meconium.
Horse 20884	15/9/44	Inoculated with collodion filtrate of foetal organ from Pinetown	Aborted on 17/10/44...	Organs of foetus positive for <i>abortus-equi</i>	Extensive haemorrhages on serous and mucous membranes. Very prominent secondary follicles, haemorrhages in lymph glands
Horse 685..	27/11/44	Inoculated with collodion filtrate of foetal organ from Pinetown	Foaled on 29/1/45. Foal weak and died on 31/1/45	Organs of foal negative for <i>abortus-equi</i>	Hydropericardium, epicardial petechiae, foetal atelectasis.
Horse 683..	4/12/44	Inoculated with collodion filtrate of foetal organs from Pinetown	Aborted on 26/12/44...	Organs of foetus negative for <i>abortus-equi</i>	Sub-epi- and sub-endo-cardial haemorrhages; petechiae on lungs and spleen; hydrothorax and ascites.
Donkey 686	14/12/44	Inoculated with collodion filtrate of the organs of the foetus of mare 691	Aborted on 17/2/45....	Foetal organs positive for <i>abortus-equi</i>	Hydropericardium, sub-epi- and sub-endo-cardial haemorrhages; tumor splenis; stasis of liver.
Donkey 692	21/12/44	Inoculated with collodion filtrate of the organs of the foetus of mare 691	Aborted a 4-5 months old foetus on 20/2/45	Foetal organs positive for <i>abortus-equi</i>	Hydro-thorax. hydro-pericardium, ascites, swelling of the spleen, icterus, oedema of lungs.
Donkey 694	21/12/44	Inoculated with collodion filtrate of the organs of the foetus of mare 691	Aborted on 25/1/45....	Foetal organs positive for <i>abortus-equi</i>	Hyperaemia of spleen and liver. Degeneration of liver.

* Foal developed joint-ill severely, from which it died later on. *Abortus-equi* isolated from fluid of all affected joints. Agglutination test of mare serum negative.

TABLE 5—(continued).

No. of Animal.	Date of entry into Experiment.	Nature of Experiment.	Result.	Result of Bacteriological Examination of Foetus or Foal.	Pathological Anatomy.
Donkey 731	13/2/45	Inoculated with candle filtrates of organs of foetuses 705 and 694	Aborted a fully grown foal on 22/2/45	Foetal organs and afterbirth negative for <i>abortus-equi</i>	Hydro-thorax, hydro-pericard., ascites, oedema of lungs, sub-epi-endocardial haemorrhages, petechiae on spleen, spleen swollen, marked hyperaemia of intestines, stasis of liver.
Donkey 726	13/3/45	Inoculated with candle filtrates of organs of foetuses 705 and 694	Foaled normally on 2/2/45 foal appears normal	Afterbirth negative	—
Donkey 724	13/2/45	Inoculated with candle filtrates of organs of foetuses 705 and 694	Foaled normally on 20/2/45; foal appears normal	Afterbirth negative for <i>abortus-equi</i>	
Donkey 729	15/2/45	Dosed with 30 c.c. candle filtrate of organs of foetuses 705 and 694	Foaled normally on 12/3/45	Afterbirth negative for <i>abortus-equi</i>	—
Donkey 732	15/2/45	Dosed with 30 c.c. candle filtrate of foetuses 705 and 694	Foaled on 22/3/45 . . .	Afterbirth positive for <i>abortus-equi</i>	—
Donkey 725	24/2/45	Dosed with 50 c.c. candle filtrate of foetuses 686 and 692	Foaled on 28/3/45	Afterbirth negative for <i>abortus-equi</i>	—
Donkey 727	24/2/45	Inoculated with collodion filtrates of organs of foetuses of 686 and 692	Aborted on 30/3/45 . . .	Afterbirth and foetal organs positive for <i>abortus-equi</i>	Moderate hydropericardium and hydro-thorax sub-endocardial haemorrhages, petechiae on spleen, tumor splenis.
Donkey 733	24/2/45	Inoculated with candle filtrate of organs of foetuses of 686 and 692	Foaled on 9/4/45	Afterbirth negative for <i>abortus-equi</i>	—

Live *abortus-equi* in broth culture or in saline emulsion failed to pass through collodion membranes (pores, 810 milli-micron.)

Filtration was also carried out by means of Berkefeld candles. Approximately 2 c.c. of the filtrate was inoculated into each of six tubes containing chopped meat broth. Although four of the tubes remained sterile after two weeks' cultivation, two tubes showed a turbidity. When the latter were subcultured on MacConkey the one yielded *B.coli* and the other a slow growing Gram-positive coccus, but no organism that resembled a salmonella could be found. These organisms were therefore also regarded as contaminants.

An attempt was made by Mr. D. Haig of the Section of Virus Diseases to cultivate the infective agent present in collodion filtrates on chorio-allantoic membranes, but without any success so far. He found, however, that, whereas chicken embryos were readily killed by means of live *abortus-equi*, nearly all the embryos inoculated with collodion filtrates developed normally.

A histological study of the morbid organs is being carried out by Mr. de Boom of the Section of Pathology and this work will form the subject of a separate paper later on. Meanwhile it can be stated that after a preliminary examination the intra-nuclear inclusion bodies described by Dimock, Edwards and Bruner (1942) have not yet been found.

1. *Horse mare* 22523 was inoculated intravenously on 17th March, 1944, with about 15 c.c. of the filtrate of foetal organs obtained from Pinetown. On 7th September, 1944, she foaled an apparently normal foal. The after-birth was negative for *abortus-equi*, but about 4 weeks later the foal developed joint-ill from which it died. *Abortus-equi* was isolated from the fluid in all the affected joints and tendon sheaths. The serum of the foal showed a strongly positive agglutination reaction, but the mare's serum was negative.

2. *Horse mare* 20810 was inoculated intravenously on 5th September, 1944, with about 15 c.c. of the filtrate of foetal organs obtained from Pinetown. On 23rd October, 1944, i.e., 48 days later, a live foal was dropped which died 24 hours later. The organs of this foal were positive for *abortus-equi* and the foal presented the following lesions: General icterus, sub-epicardial and sub-endocardial haemorrhages, oedema of lungs, hyperaemia of stomach and intestine, hyperaemia of brain and slight hydrocephalus, retention of the meconium. The serum of the mare was negative at the time of parturition and remained negative.

3. *Horse mare* 20884 was inoculated on 15th September, 1944, with the filtrate of foetal organs obtained from Pinetown and aborted on 17th October, 1944, i.e., 33 days later. All the organs of the foetus were positive for *abortus-equi*; the serum of the mare, negative at the time of abortion, was positive 17 days later. The most important lesions presented by the foetus were extensive haemorrhages on the serous membranes, very prominent secondary follicles and haemorrhages in the lymph glands.

4. *Horse mare* 685 was inoculated on 27th November, 1944, with filtrate of foetal organs obtained from Pinetown. It gave birth to a live foal on 29th January, 1945, i.e., 64 days later, but the foal was weak and died two days later, showing lesions of hydropericardium, epicardial petechiae and foetal atelectasis. The organs of the foal were all negative for *abortus-equi*. The serum of the mare was negative on the day of foaling and remained negative for more than a month.

5. *Horse mare* 683 was inoculated on 4th December, 1944, with filtrate of foetal organs obtained from Pinetown. It aborted on 26th December, 1944, i.e., 16 days later, and the foetus presented the following lesions: Sub-epi- and sub-endocardial haemorrhages, petechiae on the surface of the lungs and spleen, slight hydrothorax and ascites. The foetal organs were negative bacteriologically and the serum of the mare was negative at the time of abortion, remaining negative for at least two months.

6. *Donkey mare* 686 was inoculated on 14th December, 1944, with the filtrate of foetal organs of donkey mare 691. She aborted on 17th February, 1945, i.e., 65 days afterwards. The foetal organs were positive for *abortus-equi* and presented lesions of

TABLE 6.
Serological Reactions of the Sera of Mares in Table 5 with Abortus-Equi "O" and "H".

No. of Animal.	Date of Abortion or Foaling.	Date of Test.	O.	H.	Date of Test.	O.	H.	Date of Test.	O.	H.	Date of Test.	O.	H.
Horse 22523	Foaled on 7/9/44.....	2 9/44	40	640	15/9/44	40	160	30/9/44	20	80	3/11/44	20	40
Horse 20810	Foaled on 23/10/44. Foal died 24/10/44	4/9/44	20	10	25/10/44	20	10	30/10/44	20	20	3/11/44	20	10
Horse 20884	Aborted on 17/10/44.....	12 9/44	0	10	17/10/44	40	40	3/11/44	160	320	19/2/45	20	20
Horse 685...	Foaled on 29/1/45. Foal died 31/1/45	27/11/44	20	10	29/1/45	10	20	8/2/45	40	20	26/2/45	40	20
Horse 683...	Aborted on 26/1/45.....	27/11/44	0	20	27/12/44	0	20	15/1/45	20	20	8/2/45	20	20
Donkey 686.	Aborted on 17/2/45.....	27/11/44	40	80	19/2/45	40	160	8/3/45	160	1280	26/3/45	80	640
Donkey 692	Aborted on 20/2/45.....	27/11/44	10	40	20/2/45	40	80	10/3/45	40	1280	26/3/45	1280	320
Donkey 694	Aborted on 23/1/45.....	27/11/44	10	40	23/1/45	40	160	8/2/45	320	160	15/3/45	640	80
Donkey 731	Aborted on 22/2/45.....	13/2/45	40	20	22/2/45	40	20	5/3/45	40	20	16/3/45	40	10
Donkey 726	Foaled on 2/3/45.....	13/2/45	20	20	2/3/45	40	20	20/3/45	160	80	26/3/45	320	160
Donkey 724	Foaled on 20/2/45.....	13/2/45	20	10	20/2/45	40	20	8/3/45	20	40	26/3/45	80	80
Donkey 729	Foaled on 12/3/45.....	13/2/45	20	40	13/3/45	0	10	26/3/45	160	80	3/4/45	320	80
Donkey 732	Foaled on 22/3/45.....	13/2/45	10	0	22/3/45	20	80	3/4/45	20	0	13/4/45	80	20
Donkey 725	Foaled on 28/3/45.....	13/2/45	80	40	28/3/45	160	320	3/4/45	80	40	21/4/45	320	160
Donkey 727	Aborted on 30/3/45.....	13/2/45	20	40	31/3/45	20	80	10/4/45	40	2560	21/4/45	160	2560
Donkey 733	Foaled on 9/4/45.....	13/2/45	40	10	10/4/45	20	320	21/4/45	10	320	—	—	—

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hydropericardium, sub-epicardial and sub-endocardial haemorrhages, swelling of spleen and stasis of the liver. The serum of the mare, negative at the time of abortion, was positive 19 days later.

7. *Donkey mare 692* was inoculated on 21st December, 1944, with the filtrate of the foetal organs of donkey mare 691. She aborted a 4-5 months old foetus on 20th February, 1945, i.e., after 61 days. The foetal organs were culturally positive for *abortus-equi* and the foetus presented lesions of hydrothorax, hydropericardium, ascites, swelling of the spleen, icterus, degeneration of the liver and oedema of the lungs. The serum of the mare, negative at the time of abortion, was positive 19 days later.

8. *Donkey mare 694* was inoculated on 21st December, 1944, with the filtrate of the foetal organs of donkey mare 691. She aborted on 23rd January, 1945, i.e., after 33 days. The foetal organs were positive for *abortus-equi* and the foetus presented lesions of hyperaemia of the liver and spleen and degeneration of the kidneys. The serum of the mare was suspicious or slightly positive on the day of abortion but became strongly positive 2 weeks later.

9. *Donkey mare 731* was inoculated on 13th February, 1945, with candled filtrates of the foetuses of donkey mares 705 and 694. She aborted a fully-developed foal on 22nd February, 1945, i.e., 9 days afterwards. The foetal organs were culturally negative for *abortus-equi* and the foetus presented lesions of hydrothorax, ascites, hydropericardium, sub-epicardial and sub-endocardial haemorrhages, petechiae on surface of spleen, swelling of spleen, oedema of lungs, stasis of liver and intense hyperaemia of the intestines. The serum of the mare was negative at the time of abortion, and remained negative.

10. *Donkey mare 726* was inoculated on 13th February, 1945, with candled filtrates of foetal organs of 705 and 694. It gave birth to an apparently normal foal on 2nd March, 1945. Its after-birth was negative for *abortus-equi*. Its serum, negative on the day of parturition, was positive 18 days later.

11. *Donkey mare 729* was dosed with candled filtrates of the foetal organs of donkey mares 705 and 694 on 15th February, 1945. She foaled an apparently normal and healthy foal on 12th March, 1945, and the after-birth was negative for *abortus-equi*. Her serum was negative on the day of parturition, but positive 2 weeks later.

12. *Donkey mare 732* was dosed on 15th February, 1945, with candled filtrates of foetuses of donkeys 694 and 705. She delivered an apparently normal foal on 22nd March, 1945. The after-birth was positive for *abortus-equi*. The serum, negative at the time of abortion, was still negative 2 weeks later.

13. *Donkey mare 725* was dosed on 24th of February, 1945, with approximately 50 c.c. of candled filtrates of the organs of the foetuses of donkeys 686 and 692. Its after-birth was culturally negative for *abortus-equi*, but its serum was positive at the time of parturition.

14. *Donkey mare 727* was inoculated on 24th of February, 1945, with about 20 c.c. of collodion membrane filtrates of the organs of foetuses of donkeys 686 and 692. It aborted on the 30th of March. The organs of the foetus showed lesions of endocardial haemorrhages, petechiae on surface of spleen, tumor splenis, moderate hydropericardium and hydrothorax. The foetal organs and after-birth readily yielded *abortus-equi* on culture. The serum of the mare was negative at the time of abortion, but positive two weeks later.

All the equines used in the experiment gave negative tests for dourine. Five horse mares, Nos. 22523, 20810, 80884, 176, and 21818, were selected at random from a group of twelve pregnant mares that had been running for a number of years on Kaalplaas, a farm adjoining Onderstepoort. The five mares used in the experiment were transferred to Onderstepoort, while the remaining seven remained at Kaalplaas. The latter all gave birth to apparently normal and healthy foals, that have remained healthy for more than twelve months.

The other equines used in the experiment were obtained from an area where infectious equine abortion is not known to occur.

It was found that the mares in which abortion was produced experimentally did not suffer in any way as the result of the premature birth; the after-birth was expelled immediately after parturition and involution of the uterus took place as in a normal birth. The majority of the experimentally produced abortions occurred from 7-10 months of pregnancy, but one foetus was dropped when it was barely 5 months old while another was fully developed at the time of delivery. The incubation period also varied considerably; in one case a foetus was expelled 9 days after the mare was inoculated with filtrate, whereas in three other cases the abortion took place after the lapse of over 60 days (61, 64 and 65 days respectively). As a rule the incubation period varied from 15 to 33 days; it appeared to be shorter in the mares given minced organs by the mouth than in those inoculated with filtrate—probably because the former received far more infective material.

The virulence of the infecting agent appeared to decrease during the end of an epizootic. In the Smal outbreak only two live foals were produced by 29 mares during a period of 10 weeks whereas three of the last five mares that carried their foals till the end of the outbreak delivered live foals, only two abortions occurring. At Onderstepoort nearly a 100 per cent. abortions were produced during the first part of the experiment, while the majority of donkey mares subsequently infected by means of either filtrates or unfiltered material gave birth to live foals.

In the Smal outbreak the disease ran its course during one season (1942) with an abortion incidence of over 85 per cent. and then completely disappeared. During the subsequent breeding seasons (1943 and 1944) not a single abortion was reported.

At present it cannot be stated whether the disappearance of the disease should be attributed to the immunity resulting from the infection or to the attenuation of the virus during the course of the outbreak.

SUMMARY OF RESULTS.

1. (See Tables 3 and 4.) Three horse mares, Nos. 176, 21818 and 682, and two donkey mares, Nos. 691 and 728, were dosed with minced foetal organs infected with *abortus-equi*. All three the horse mares aborted after an incubation period varying from 16 to 37 days; one donkey mare (691) aborted after 15 days, the other (728) turned out not to be pregnant.

The foetuses of all three horse mares and of the one donkey mare were infected with *S. abortus-equi*.

The sera of the three horse mares and one donkey mare, No. 691, were negative when abortion occurred but became positive within two weeks.

Two donkey mares, Nos. 704 and 705, were dosed with the minced foetal organs of horse mare 683 which were culturally free from *abortus-equi* infection. The one, No. 705, aborted after 21 days, while the other one, No. 704, gave birth to a live foal after 42 days. The foetal organs of 705 and the after-birth of 704 were infected with *S. abortus-equi*. The serum of 705 was negative at the time of abortion and positive 14 days later, but the serum of 704 was negative at the time of parturition and remained negative.

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Three donkey mares, Nos. 693, 697 and 689, were dosed with large amounts of live *abortus-equi* culture. The one, No. 693, gave birth to an apparently normal foal after 23 days, while the foal of another mare, No. 697, died soon after birth, apparently as a result of dystokia. No. 689 gave birth to a healthy foal after 118 days. The organs of the foal of No. 697 were culturally negative for *abortus-equi* and the mare's serum was also negative. The serum of 693, negative at the time of parturition, was positive about two weeks later, while the serum of 689 was positive at the time of parturition.

The lesions presented by the foetal organs of horse mares 176, 21818 and 682 and of donkey mares 691 and 705 were typical of those found in the naturally occurring disease but no definite lesions could be found in the foal of donkey mare 697, which was dosed with live culture.

One donkey mare, No. 687, was dosed with the infected synovial fluids of the foal of mare 22523 which was suffering from joint-evil. It foaled apparently normally after 102 days. Its after-birth was culturally negative for *abortus-equi* and its serum was also negative.

2. (See Tables 5 and 6.) Five horse mares, Nos. 22523, 20810, 20884, 685 and 683, and six donkey mares, Nos. 686, 692, 694, 688, 727 and 730, were inoculated intravenously with collodion filtrates of *abortus-equi* infected foetal organs.

Horse mares, Nos. 20884 and 683, and donkey mares 686, 692, 727 and foal of 22523 developed *abortus-equi* infected joint-ill from which it died. The foal of 20810 lived for 24 hours and the foal of 685 for about 48 hours; on post mortem both presented lesions which are typical of infectious abortion, the organs of the former being culturally positive for *abortus-equi* but those of the latter negative. The sera of all three mares were negative at the time of parturition and remained negative thereafter.

Horse mares, Nos. 20884 and 683, and donkey mares 686, 692, 727 and 694 aborted and all their foetuses presented lesions which resemble those found in the naturally occurring disease. The organs of the foetuses of horse mare 20884 and donkey mares 686, 727, 692 and 694 were culturally positive for *abortus-equi*, but the organs of the foetus of 683 were negative. The sera of all six mares were negative at the time of abortion and became positive in the case of 20884, 686, 692, 694, and 727, but the serum of 683 remained negative.

Donkey mares 731, 726, 724 and 733 were inoculated intravenously with candle filtrates of *abortus-equi* infected foetal organs, while Nos. 729, 732 and 725 were dosed with similar filtrates. No. 724 gave birth to a live foal on the 7th day while No. 731 aborted after 9 days; Nos. 726, 732, 725 and 729 also produced live foals. The lesions presented by the foetus of 731 were typical of infectious abortion but the foetal organs were all negative for *abortus-equi*. *Abortus-equi* was recovered from the after-birth of No. 732 (and also from 704), but not from Nos. 724, 729, 731, 725 and 726. The sera of all these mares were negative at the time of parturition or abortion and remained negative, except in the case of 726, whose serum was positive 18 days after parturition.

The preliminary results obtained with eight guinea-pigs and one mare in 1942 when foetal material from the Smal outbreak was used, are not given here.

DISCUSSION.

In the evidence presented in the review of the literature it is apparent that the etiology of infectious (epizootic) equine abortion requires elucidation. Most workers agree that a bacterium, *S. abortus-equi*, is the most important etiological agent, but during recent years a number of investigators have incriminated a virus as the cause of some outbreaks of abortion. Thus Dimock and Edwards (1936), Miessner (1938) and Hupbauer (1938), although recognising the importance of *abortus-equi* as the cause of the majority of epizootics, were unable to find this organism in the foetal organs examined by them during some outbreaks and incriminated a virus.

On the other hand several other investigators like Kilborne (1893), Smith (1893), Good and Corbett (1913), Van Heelsbergen (1914), Schofield (1914), MacFadyean and Stockman (1917), Murray (1919), Verge (1939), and Saxer (1938) have isolated *abortus-equi* from the majority of aborted foetuses examined during the outbreaks investigated by them. During the transmission experiments carried out by some of these workers it was possible to produce abortion only when live cultures of the organism were inoculated intravenously or intraperitoneally and, as the abortion occurred after a comparatively short incubation period, it was probably the result of an *abortus-equi* septicaemia. Good and Corbett (1913) admit that the experimental mare may be off colour for a few days as a result of the inoculation. Premature birth could not be produced when the cultures were given by the mouth or per vaginam.

Van Heelsbergen (1914), Hupbauer (1938), and others, although recommending the immunization of pregnant mares with killed cultures of *abortus-equi*, admit that this form of immunization will not protect against exposure to infection. Notwithstanding this, dead cultures have been found to produce a very high agglutination titre in inoculated animals (Table 1).

The fact that infected mares do not abort more than once, and usually give birth to healthy foals during succeeding pregnancies, is an indication that they have had an immunity conferred upon them as a result of the previous natural infection. Because this infection may be associated with invasion of the tissues by *abortus-equi* it is no proof that this organism is the cause of the abortion, nor does it follow that *abortus-equi* has conferred the resistance.

The disease reported by Dimock and Edwards (1936), Miessner (1938), and Hupbauer (1938), has so many features in common with the one described by Good and Corbett (1913), van Heelsbergen (1914), Murray (1919), and others, that they cannot be differentiated on clinical and pathological grounds. They both showed a number of common features, viz., the infection is extremely contagious, the abortion incidence is very high (up to 90 per cent.), the disease appears suddenly during one season and usually does not recur in the same stud during two consecutive years, the affected mare apparently suffers no ill-effect and usually takes the stallion as readily as an unaffected mare.

On the other hand, it should be pointed out that we have not yet succeeded in demonstrating the presence of the "inclusion bodies" described by Dimock, Edwards and Bruner (1942).

In the experiments reported here abortion was readily provoked in both horse and donkey mares by means of filtered and unfiltered suspensions of infected foetal organs, but it was not possible to induce abortion when

large doses of live *abortus-equi* cultures were administered by the mouth. All the aborted foetuses presented the same lesions, whether the mare had received filtered or unfiltered organ suspensions.

The question arises, therefore, what rôle *abortus-equi* plays in the etiology of infectious equine abortion. Of the five horse mares that were inoculated with filtrate two aborted and three dropped live foals. Of the latter, two were so weak that they died within 48 hours and presented lesions which are typical of the disease; *abortus-equi* was recovered from the one, but not from the other foal; the third foal developed joint-ill from which it died. When the *abortus-equi*-free, unfiltered organs of the foetus of horse mare 683 were given to two donkey mares (704 and 705), the one (705) aborted, while the other (704) gave birth to a live foal. But, notwithstanding the absence of *abortus-equi* in the foetal organs dosed, this organism was recovered in large numbers from the organs of the aborted foetus of 705 and in smaller numbers from the after-birth of 704. *Abortus-equi* was also recovered from the after-birth of another mare (732) that had delivered a live foal.

In spite of their apparently greater resistance to infectious equine abortion five of the donkey mares inoculated with filtrate aborted; in the case of four, collodion membrane filtrates were used and in the other one Berkefeld candle filtrates. The foetal organs of all five presented typical lesions of equine abortion. *Abortus-equi* was recovered from the organs of the first four foetuses but not from the last.

When the serological results (Tables 1, 2, 4 and 6) are studied it will be noticed that, if the organs of an aborted foetus have been invaded by *abortus-equi*, the serum of the mare, negative at the time of abortion, invariably becomes positive in about two weeks. If the foetal organs are free from *abortus-equi* the agglutination reaction is likely to remain negative.

When a live foal has been born and it dies within 48 hours the serum of the mare generally remains negative whether the foetal organs have been invaded or not. (See agglutination reactions of mares 20810 and 685.)

Several workers have published information which shows that *abortus-equi* may occur in the body of equines as a secondary invader. Thus, Stitz and Görkel (1938) isolated this organism from the faeces, urine and uterine discharges of horses; they also obtained it from a variety of diseases in which it could not be associated with the clinical picture or the lesions. Geldings as well as mares were found to be carriers. Sometimes *abortus-equi* invades the tissues and may be associated with suppurations and abscessation—Martinaglia (1929), Good and Corbett (1913), Good and Smith (1914), Schofield (1914), MacFadyean and Stockman (1917), Miessner and Berge (1917), Watanabe (1937), Oguni and Koihutsu (1938).

In the experiments reported here *abortus-equi* sometimes was found to invade the tissues of a foetus when the mare had aborted after receiving bacteria-free filtrates, whereas in other cases the organism could not be recovered from the aborted foetus when the mare was inoculated with a similar filtrate. Sometimes *abortus-equi* was obtained from the after-birth of a mare which has delivered a live foal (704 and 732). The fact that the serum of a mare is usually negative at the time of abortion but positive 2 weeks later, provided the foetus is invaded, is an indication that the tissues of the mare have not been entered long before the abortion occurred.

It would appear, therefore, that the rôle played by *abortus-equi* in relation to infectious equine abortion is analogous to that of *S. cholerae-suis* in relation to hog-cholera. For many years after the discovery by Salmon and Smith (1885) of *S. cholerae-suis* in the morbid tissues of pigs that had died from swine fever this organism was universally regarded as the cause of the disease. Later, however, de Schweinitz and Dorset (1903), and Dorset, Bolton and McBryde (1904), showed beyond doubt that a filterable virus is the real cause and that *S. cholerae-suis* serves merely as a secondary invader, entering the tissues only after the virus has lowered the resistance of the body.

On the other hand, it may be possible that, as with *Haemophilus influenzae suis* and swine influenza virus (Bang, 1943), there is a synergistic action between *S. abortus-equi* and the filterable infecting agent of equine abortion.

The results reported here clearly indicate (1) that the infective agent of infectious equine abortion is present in the morbid tissues of an aborted foetus and that it is filterable through collodion membrane (pores, 810 millimicra) and Berkefeld candles; (2) that *abortus-equi* may or may not be present in the infected foetal tissues and (3) that live cultures of *abortus-equi*, when given alone, cannot produce abortion, whereas bacteria-free filtrates of infected organs generally cause premature birth.

The fact that *abortus-equi* is present in the morbid organs of the majority of the aborted fetuses studied, though not in all, cannot be accepted as proof that it is the cause of infectious equine abortion. Like *S. cholerae-suis* it should be regarded as a secondary invader, which frequently enters the tissues of the foetus and the foetal membranes after their resistance has been lowered by the filterable infective agent which is the primary cause.

The association of a salmonella as a secondary infective agent with the primary cause (a virus) of two epizootic diseases, viz. swine-fever and infectious equine abortion, suggests the advisability of further investigating the etiological significance of other salmonellas found in epizootic diseases like calf paratyphoid and fowl typhoid.

SUMMARY AND CONCLUSIONS.

1. Two outbreaks of infectious equine abortions are reported; both with a very high abortion incidence.
2. In the majority of the abortions studied the foetal organs were found to be extensively invaded by *S. abortus-equi* but in others this organism could not be recovered from the foetus or after-birth.
3. Both donkey and horse mares were found to be susceptible, donkeys being on the whole more resistant than horses.
4. Abortion was successfully produced in both horse and donkey mares by means of (a) the oral administration of minced *abortus-equi* infected foetal organs (4 cases), (b) intravenous inoculation of collodion membrane or Berkefeld candle filtrates of these organs (9 cases, including 2 foals that died within 48 hours of birth) and (c) the oral administration of minced foetal organs that were culturally free from *abortus-equi* (one case).
5. The twelve aborted fetuses and the two foals that died within 48 hours of birth all presented lesions typical of infectious equine abortion.

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6. *Abortus-equi* was recovered from the organs of ten of the aborted foetuses and one of the dead foals, but not from two aborted foetuses and one dead foal. It was also isolated from the after-birth of two mares that had given birth to live foals (Nos. 704 and 732).

7. All the mares gave negative agglutination reactions at the time of abortion or parturition, but in those cases where *abortus-equi* was recovered from the foetus—not the foal—the reaction became positive in about 2 weeks. When the organs of the foetus were free from *abortus-equi* or when a live foal was born, whether its organs contained *abortus-equi* or not, the agglutination reaction of the mare generally remained negative.

8. The aborting mares did not suffer any ill effects as the results of the abortions and the after-births were expelled normally.

9. It is concluded that the primary cause of infectious equine abortion is an infecting agent that will pass either through collodion membrane, with the size of the pores 810 milli-micra, or through Berkefeld candles.

10. It was not possible to produce abortion in three pregnant donkey mares dosed with large amounts of live *abortus-equi* culture.

11. The significance of *S. abortus-equi* in equine abortion is comparable with that of *S. cholerae* in swine-fever. Like *cholerae-suis*, *abortus-equi* is regarded as a saprophyte which frequently occurs in the body of the horse without causing any obvious disturbance, invading the tissues of the body only when conditions become favourable. When the resistance of the foetus, foetal membranes and uterus has been lowered by the primary cause of infectious abortion, viz., the filterable infecting agent, this organism enters and causes a secondary infection. Alternatively *abortus-equi* plays the role of a synergist as *H. influenzae suis*.

12. The advisability of further investigating the etiological significance of salmonella encountered in epizootic diseases like calf paratyphoid and fowl typhoid is suggested.

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Avirulent Anthrax Vaccine.

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A FEW years ago a report was published on field tests with anthrax vaccines made from avirulent uncapsulated strains [Sterne (1939)]. The vaccine was subsequently brought into regular use, and at present more than six million doses are used annually. Altogether, more than 30 million doses have been used.

The results have equalled expectations and far surpass those obtained with spore vaccines of the Pasteur type. In the last three years, for example, during which more than fifteen million animals have been inoculated, there have been only twenty complaints to the laboratory.

Four (1) were outbreaks of *Cl. chauvoei* infection following vaccination.

Four (4) were of deaths following inoculation. In all, about 14 animals died. The association of inoculation and death was fortuitous.

Ten (10) complaints were of abscesses, swellings, or severe reactions following inoculation.

Two (2) complaints were of insufficient immunity. In one case a farmer lost two animals two months after immunization. The description of the post mortem made it fairly clear that the deaths were not due to anthrax. Another farmer lost an animal two months after immunization. This was definitely anthrax.

It is not suggested that every misfortune is reported. Complaints are, however, a small fraction of the number we used to get.

PREPARATION OF THE VACCINE.

Apart from slight modifications, the method of obtaining avirulent variants is the same as that described in earlier papers [Sterne (1937a, 1937b)]. The strains are preserved by drying in the frozen state. Stock cultures are prepared from the ampoules of dried spores and kept on agar slants under sterile liquid paraffin.

A batch of vaccine is prepared by sowing a broth suspension of 24-hour agar cultures on to Woodhead flasks of Gladstone and Fildes' (1940) casein hydrolysate agar. We simply mix the hydrolysate, the tryptic digest of casein, and the yeast brew with water and agar. Nothing else is added. This medium is very simple to prepare, very cheap and very satisfactory. Sporulation is usually complete after 48 hours and the batch is harvested on the third day by washing off with physiological saline. Twice the amount by weight of glycerine is then added. This stock suspension is adjusted to contain 6×10^8 spores per cubic centimetre. The immunizing power is tested on guinea-pigs as follows:—

AVIRULENT ANTHRAX VACCINE.

Six (6) guinea-pigs receive 0·01 c.c., and six (6) guinea-pigs 0·001 c.c. of the stock suspension subcutaneously on the abdomen. Three weeks later, these guinea-pigs, together with six controls receive 0·1 c.c. of a glycerine-saline spore suspension of a Pasteur II strain subcutaneously in the hind leg. This dose of the Pasteur strain contains about 100 guinea-pig lethal doses. It is important that the test should be given well away from the site of the immunizing dose to avoid non-specific effects. If the guinea-pigs immunized with the 0·01 c.c. dose of vaccine resist the test dose, the vaccine is passed for a field test. The field test is merely a precaution. Several hundred animals are injected with a dose of the vaccine four times the strength of that finally issued. So far no batch has failed in this test.

Below is a typical laboratory test. It is an average test; neither very good nor very bad. Duplicate titrations of the same batch agree reasonably well.

BATCH 15.

No. of Guinea Pig.	Amount of Concentrated Vaccine.	Date.	Result.	Amount of Test Dose (100 M.I.D.)	Date.	Result.
1.....	0·01 c.c.	22/11/43	L	0·1 c.c.	11/12/43	L
2.....	0·01 c.c.	22/11/43	L	0·1 c.c.	11/12/43	L
3.....	0·01 c.c.	22/11/43	L	0·1 c.c.	11/12/43	L
4.....	0·01 c.c.	22/11/43	L	0·1 c.c.	11/12/43	L
4.....	0·01 c.c.	22/11/43	L	0·1 c.c.	11/12/43	L
5.....	0·01 c.c.	22/11/43	L	0·1 c.c.	11/12/43	L
6.....	0·01 c.c.	22/11/43	L	0·1 c.c.	11/12/43	L
7.....	0·001 c.c.	22/11/43	L	0·1 c.c.	11/12/43	L
8.....	0·001 c.c.	22/11/43	L	0·1 c.c.	11/12/43	L
9.....	0·001 c.c.	22/11/43	L	0·1 c.c.	11/12/43	L
10.....	0·001 c.c.	22/11/43	L	0·1 c.c.	11/12/43	L
11.....	0·001 c.c.	22/11/43	L	0·1 c.c.	11/12/43	†2*
12.....	0·001 c.c.	22/11/43	L	0·1 c.c.	11/12/43	†2
13.....	Nil	—	—	0·1 c.c.	11/12/43	†2
14.....	Nil	—	—	0·1 c.c.	11/12/43	†2
15.....	Nil	—	—	0·1 c.c.	11/12/43	†2
16.....	Nil	—	—	0·1 c.c.	11/21/43	†2
17.....	Nil	—	—	0·1 c.c.	11/12/43	†2
18.....	Nil	—	—	0·1 c.c.	11/12/43	†3

L - Lived.

* † 2 = dead by second day.

Before issue, the vaccine is diluted $\frac{1}{50}$ with 50 per cent. glycerine-saline and $\frac{1}{2}$ per cent. Evan's saponin or $\frac{1}{2}$ per cent. Merck's saponin. The strengths of different brands of saponin can be compared by injecting serial dilutions intra-dermally into guinea-pigs. The dose of vaccine used is one cubic centimetre, containing about 10 million spores.

Response of different animals to the vaccine.

The same vaccine is now used for all animals. Cattle and sheep react very mildly. Horses react more vigorously; but no farmer has yet complained about the reactions. Goats, in general, show little reaction; but now and then may show large swellings, and deaths have been reported. Work

done on guinea-pigs indicates that the oedema-producing property of our strains is not entirely a function of the immunizing power, and that the medium used is an important factor. The casein medium has proved the least provocative in this respect.

Guinea-pigs develop a good immunity within a few days, and field results indicate that the response of cattle is equally fast. In horses, on the other hand, a sound immunity takes a month to six weeks to develop.

SUMMARY AND CONCLUSIONS.

More than 30 million doses of anthrax vaccine made from avirulent unencapsulated variants have been used in South Africa. The same vaccine is used for all domestic animals, and the results far surpass those obtained with the Pasteur type of spore vaccine. The strains sporulate very readily, and their immunizing power is easily tested in guinea-pigs. It has been found possible to dispense with large-animal immunity tests. Thus these strains lend themselves very well to laboratory manipulations.

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Recent Investigations into the Toxicity of Plants, Etc., No. XV.

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APOCYNACEAE.

Vinca major L.

Registered number.—O.P.H. 13236; 11.10.43.

Common name.—Periwinkle.

Origin.—Pretoria, Transvaal.

State and stage of development.—The plant was in the fresh state and in the flowering stage.

Sheep 67438 (6-tooth; 36.4 Kg.) was given* 3.7 Kg. of the plant in the course of 4 days (12.10.43-16.10.43).

Symptoms.—Every time after being drenched the sheep developed tympanites. On 14.10.43 such a severe tympanites had developed after the sheep was drenched in the morning that it was considered inadvisable to drench the animal in the afternoon. On 15.10.43 the following was observed: anorexia; apathy; no defaecation; dyspnoea; accelerated strong pulse; fair degree of tympanites. On 16.10.43 the condition of the animal was unchanged. The animal died overnight on 16.10.43.

Post-mortem appearances.—Too decomposed for examination.

Sheep 67550 (6-tooth; 34.1 Kg.) was given 6.0 Kg. of the plant in the course of 11 days.

Result.—Negative.

From the above it would appear that sheep 67438 died of digestive disturbances as a result of being drenched with the plant material.

* Except where otherwise stated all the animals were drenched by means of a stomach tube.

AIZOACEAE.

Tetragonia Schenkii Schinz. (Fig. 1.)

Registered number.—O.P.H. No. 3666; 22.5.43.

Common name.—Koibos.

Origin.—Windhoek, South West Africa.



FIG. 1.—*Tetragonia Schenkii* Schinz.

State and stage of development.—The plant was in the dry state without flowers or fruit.

Sheep 66463 (4-tooth; 26·9 Kg.) was given 500 gm. of the plant in the course of 7 hours.

Symptoms.—The animal died overnight on the day of drenching.

Post-mortem appearances.—Slight post-mortem changes; general cyanosis; ascites; hydropericardium; subepicardial petechiae; hyperaemia, oedema and emphysema of the lungs; regressive changes in the liver; tympanites of the rumen; slight hyperaemia of the mucosa of the abomasum, jejunum and colon.

Sheep 66304 (6-tooth; 31·9 Kg.) was given 250 gm. of the plant in one dose.

Symptoms.—Listlessness; general weakness; anorexia; rumen inactive; slight tympanites; dyspnoea; weak pulse. The animal died overnight on the day of drenching.

Post-mortem appearances.—Severe general cyanosis; abdomen distended; ascites; subepicardial haemorrhages; a large amount of froth in the air passages; severe hyperaemia and oedema of the lungs; hydronephrosis of, and urinary calculi in, both kidneys; tympanites of the rumen.

CHENOPODIACEAE.

Beta vulgaris L.

Registered number.—O.P.H. 11422-25; 16.9.43 and 11540; 16.9.43.

Common name.—Mangels.

Origin.—Kokstad, Cape Province.

The roots of the plant, which were quite fresh, were tested.

Sheep 64549 (6-tooth; 31·9 Kg.) was given 2·4 Kg. of the first consignment of the roots (11422-25; 16.9.43) in the course of 7 hours.

Symptoms.—The animal died overnight on the day of drenching.

Post-mortem appearances.—Slight post-mortem changes; general cyanosis; slight hydrothorax; severe hydropericardium; hyperaemia of the mucosa of the trachea and bronchi; severe hyperaemia, oedema and emphysema of the lungs; subepicardial petechiae; slight regressive changes in the myocardium; severe regressive changes in the liver and kidneys; haemorrhagic lymphadenitis; slight hyperaemia of the mucosa of the abomasum, small and large intestine with sub-mucosal haemorrhages in the small intestine; stasis of the ingesta in the large intestine.

Histology.—*Liver.*—The sinusoids are distended with a large quantity of blood. The hepatic cells show parenchymatous degeneration.

Kidney.—Hyperaemia accompanied by haemorrhages whilst the proximal convoluted tubules show severe degenerative changes. The nuclei of the epithelial cells of these tubules show pyknosis and karyolysis.

Sheep 66631 (6-tooth; 34.1 Kg. was given 7.2 Kg. of the first consignment of the roots (11422-25; 16.9.43) in the course of 4 days.

Result.—Negative.

Sheep 66692 (6-tooth; 31.9 Kg.) was given 13.2 Kg. of the second consignment of the roots (11540; 16.9.43) in the course of 5 days.

Result.—Negative.

The roots were submitted for investigation in view of the fact that some of the animals feeding on the roots died rather suddenly. The results obtained in the case of sheep 66631 and 66692 indicate that the roots are non-toxic. It is considered that sheep 64549 died as the result of digestive disturbances following on the administration of relatively very large quantities of the roots.

COMPOSITÆ.

Arctotis stoechadifolia Berg.

Registered number.—O.P.H. 6896-97; 6.12.43.

Origin.—Vryburg, Cape Province.

State and stage of development.—The plant was in the dry state and in the flowering stage.

Sheep 68687 (full-mouth; 40.5 Kg.) was given 5.7 Kg. of the plant in the course of 11 days.

Result.—Negative.

Senecio glutinosus Thb.

Registered number.—O.P.H. 7239; 21.7.43.

Origin.—Pretoria, Transvaal.

State and stage of development.—The plant was in the dry state and in the flowering stage.

Sheep 64546 (6-tooth; 32.3 Kg.) was given 4.6 Kg. of the plant in the course of 6 days.

Result.—Negative.

CYCADACEÆ.

Encephalartos Lehmanni (E. & Z.) Lehm. (Fig. 2.)

Registered number.—O.P.H. No. 16422; 1.12.43.

Origin.—Division of Botany, Pretoria, Transvaal.

The fresh ripe fruit was tested. The stones were removed from the fruit in order to test the flesh and stones separately. The shells were removed from the stones.

Rabbit A (1.75 Kg.) was given 195 gm. of the flesh of the fruit in the course of 7 days.

Result.—Negative.

Rabbit B (1.6 Kg.) was given 30 gm. of the kernels in the course of 6 hours.

Symptoms. The rabbit died overnight on the day of drenching.

Post-mortem appearances. Severe oedema, emphysema and slight hyperaemia of the lungs; hyperaemia of, and regressive changes in, the liver; submucosal haemorrhages in the stomach; hyperaemia of the mucosa of some parts of the small intestine.

Rabbit C (1.85 Kg.) was given 35 gm. of the kernels in the course of 3 days.

Symptoms.—Listlessness; anorexia; dyspnoea; strong rapid pulse. The symptoms developed on the third day after the commencement of drenching and the rabbit died the same night.

Post-mortem appearances.—Hyperaemia; oedema and emphysema of the lungs; general icterus; severe ascites; severe regressive changes in the liver; regressive changes in the kidneys; hyperaemia of the mucosa of parts of the small intestine; fluid material in the colon.



FIG. 2 *Encephalartos Lehmanni* (E. & Z.) Lehmann

DICHAPETALACEAE.

Dichapetalum venenatum Engl. & Gilg.

Registered number.—O.P.H. No. 810; 19.4.44.

Common name.—Blaargif, Makou.

Origin.—Grootfontein, South West Africa.

State and stage of development.—The plant was in the dry state without flowers or fruit.

Rabbit A (2·3 Kg.) was given 10 gm. of the plant in the course of 6 hours.

Result.—Negative

Rabbit B (2·3 Kg.) was given 20 gm. of the plant in the course of 6 hours.

Symptoms.—The animal died overnight on the day of drenching.

Post-mortem appearances.—General cyanosis; severe hyperaemia of the lungs; hyperaemia of the liver and kidneys; slight hyperaemia of the mucosa of the stomach.

EBENACEAE.

Royena decidua Burch (= *R. Pallens* Thb.)

Registered number.—O.P.H. 15313; 15.11.43.

Common name.—Bloubos.

Origin.—Middelburg, Cape Province.

State and stage of development.—The plant was in the *fresh* state and in the pre-flowering stage.

Sheep 66691 (full-mouth; 41·0 Kg.) was given 1·7 Kg. of the *fresh* leaves of the plant in the course of 54 hours.

Symptoms.—Apathy; anorexia; slight dyspnoea; pulse somewhat accelerated; slight tympanites; rumen inactive; very severe diarrhoea. The animal recovered.

Sheep 66684 (6-tooth; 34·1 Kg.) was given 900 gm. of the *dry* leaves of the plant in the course of 24 hours.

Symptoms.—As for the previous sheep. The animal recovered.

IRIDACEAE.

Moraea trita var. *foliata* N.E. Br.

Registered number.—O.P.H. 12315; 25.9.43.

Common name.—Tulp.

Origin.—Pietersburg, Transvaal.

State and stage of development.—The plant was in the *fresh* state and in the flowering and seeding stages.

Sheep 66692 (6-tooth; 31 Kg.) was given 1·5 Kg. of the bulbs of the plant in the course of 24 hours.

Symptoms.—Listlessness; dyspnoea; pulse fairly slow and very strong; rumen inactive; anorexia; conjunctivae dark red. The animal died approximately 30 hours after the commencement of drenching.

Post-mortem appearances.—Advanced post-mortem changes; general cyanosis; subendocardial petechiae; hyperaemia of the mucosa of the trachea and bronchi; hyperaemia, oedema and emphysema of the lungs; tympanites of the rumen; fluid material in the large intestine; hyperaemia of certain lymphatic glands.

LEGUMINOSAE.

Bauhinia Galpini N.E. Br.

Registered number.—O.P.H. No. 9294; 18.8.43 and 11762-71; 20.9.43.

Origin.—Burberton, Transvaal.

State and stage of development.—The two consignments of the plant were without flowers or fruit. The first consignment (9294; 18.8.43) was fresh and the second (11762-71; 20.9.43) was dry.

Sheep 66849 (4-tooth; 34.1 Kg.) was given 1.1 Kg. of the fresh leaves of the first consignment of the plant in the course of 6 hours.

Result.—Negative.

Sheep 66558 (2-tooth; 25.0 Kg.) was given 9.25 Kg. of the dry leaves of the second consignment of the plant in the course of 25 days.

Result.—Negative.

Sheep 62924 (4-tooth; 34.0 Kg.) was given 4.5 Kg. of the dry leaves of the second consignment of the plant in the course of 10 days.

Result.—Negative.

Cassia Occidentalis L.

Registered number.—O.P.H. No. 11427-28; 13.9.43.

Origin.—Burao, British Somaliland.

State and stage of development.—The plant was in the dry state and in the seeding stage.

Sheep 62924 (6-tooth; 31.9 Kg.) was given 14.0 Kg. of the plant in the course of 17 days.

Symptoms.—Towards the end of the period of drenching the sheep developed digestive disturbances. The rumen became inactive and tympanites developed every time after drenching. Finally a slight diarrhoea developed. The sheep recovered immediately drenching was discontinued.

The symptoms would appear to be simply due to digestive disturbances as a result of being drenched and not due to the plant being toxic.

Medicago sativa L.

Registered number.—O.P.H. No. 8678; 9.8.43.

Common name.—Lusern, lucerne.

Origin.—Port Elizabeth, Cape Province.

The lucerne hay was infected with the following fungi: *Mucor* sp., *Aspergillus* sp., *Penicillium* sp. and *Fusarium moniliforme*.

Sheep 64549 (full-mouth; 31.9 Kg.) was given 1.69 Kg. of the infected hay in the course of 4 days.

Result.—Negative.

Phaseolus vulgaris L.

Registered number.—O.P.H. No. 66491; 18.12.43.

Origin.—Machadodorp, Transvaal.

State and stage of development.—The plant was in the fresh state and in the seeding stage.

Sheep 66491 (6-tooth; 40·5 Kg.) was given 2·01 Kg. of the corms of the plant in the course of 24 hours.

Symptoms.—The sheep developed a mild diarrhoea which lasted two days after which the animal recovered. The diarrhoea was apparently simply due to derangement of digestion as a result of drenching.

LILIACEAE.

Ornithogalum pretoriense Baker.

Registered number.—O.P.H. No. 14573; 29.10.43.

Origin.—Pretoria, Transvaal.

State and stage of development.—The plant was in the fresh state and in the flowering and seeding stages.

Sheep 67550 (full-mouth; 40·5 Kg.) was given 3·5 Kg. of the bulbs of the plant in the course of 2 days.

Result.—Negative.

Schizocarphus nerrosus (Burch.) F. v. d. M. (—*Scilla rigidifolia*
Kunth. var. *nerrosa* Bak.) (Fig. 3.)

Registered number.—O.P.H. No. 12085; 21.9.43.

Origin.—Rust-der-Winter, Transvaal.

State and stage of development.—The plant was in the fresh state and in the flowering stage.

Sheep 66461 (6-tooth; 34·1 Kg.) was given 1·0 Kg. of the plant in one dose.

Symptoms.—Severe tympanites; rumen inactive; dyspnoea; accelerated, strong pulse; apathy; anorexia. The sheep died overnight on the day of drenching.

Post-mortem appearances.—The cadaver was too decomposed for examination.

Sheep 64607 (full-mouth; 26·0 Kg.) was given 500 gm. of the plant in one dose.

Symptoms.—General weakness; apathy; fair degree of tympanites; rumen inactive; anorexia; dyspnoea; pulse very slow, strong and irregular.

Post-mortem appearances.—General cyanosis; hydropericardium; hydrothorax; ascites; subendocardial and subepicardial petechiae; slight regressive changes in the myocardium; hyperaemia and oedema of the lungs; regressive changes in the liver; tympanites of the rumen; slight hyperaemia of the mucosa of the abomasum.



FIG. 3.—*Schizocarpus nervosus* (Burch.) F.v.d.M.

Scilla iniquata C. A. Sm.

Registered number.—O.P.H. No. 12084; 21.9.43.

Origin.—Rust-der-Winter, Transvaal.

State and stage of development.—The plant was in the fresh state and in the seeding stage.

Sheep 66866 (4-tooth; 27.3 Kg.) was given 1.8 Kg. of the plant in the course of 19 hours.

Symptoms.—Following on the first dose the sheep developed a severe tympanites. At the time the second dose was given the sheep had apparently recovered. Very shortly after being drenched for the second time the animal again developed a very severe tympanites dying 4 hours after the administration of the second dose.

Post-mortem appearances.—General cyanosis; ascites; hydrothorax; hydropericardium; hyperaemia of the mucosa of the trachea and bronchi; severe hyperaemia, oedema and emphysema of the lungs; slight recessive changes in the myocardium; hyperaemia of, and regressive changes in, the liver and kidneys; severe tympanites of the rumen.

From the above it would appear that digestive disturbances, as a result of the animal being drenched, was the cause of death.

PAPAVERACEAE.

Papaver aculeatum Thunb.

Registered number.—O.P.H. No. 16824-25; 4.12.43.

Origin.—Pretoria, Transvaal.

State and stage of development.—The plant was fairly dry and in the flowering and seeding stages.

Sheep 66786 (6-tooth; 35.5 Kg.) was given 2.85 Kg. of the plant in the course of 8 days.

Result.—Negative

SOLANACEAE.

Solanum tuberosum L.

Registered number.—O.P.H. No. 16991; 9.12.43.

Common name.—Aartappel, potato.

Origin.—Vaaltakkie, Transvaal.

State and stage of development.—The potato-tops tested, were in the fresh state and in the pre-flowering stage. At first the potato-tops were fed to the animals but later, as they became drier, were minced and drenched to the animals.

Sheep 66521 (6-tooth; 30.5 Kg.) and *sheep* 68617 (2-tooth; 31.9 Kg.) were given 2.8 Kg. and 3.85 respectively of the potato-tops in the course of 9 days.

Result.—Negative.

Melasina circophora Meyr.

The pupae of this insect, commonly known as "grashuisies", were suspected to be poisonous. After removal of the pupal cases the pupae were minced and administered to a rabbit per stomach tube.

Rabbit A (1.5 Kg.) was given 30 gm. of the pupae in the course of 6 hours.

Result.—Negative.

SUMMARY AND CONCLUSIONS.

Of the 18 plants investigated the following four plants were, according to the literature available to the authors, for the first time proved to be toxic: *Tetragonia Schenkii* Schinz., *Encephalartos Lehmannii* (E. & L.) Lehm., *Moraea trita* var. *foliata* N.E. Br., and *Schizocarphus nervosus* (Burch.) F. v. d. M.

The toxicity of the pupae of *Melasina circophora* Meyr. was also investigated.

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The Biological Values of the Proteins of Some South African (Whole Seed) Maize Varieties.

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THE importance of maize in the nutrition of farm animals and of human beings in this country makes full information concerning its nutritive value essential. In a recent paper Crawford *et al* (1942) stressed the paucity of our knowledge on the chemical composition of foodstuffs grown under South African conditions and reported amongst other things that the protein content varied from 8.13 to 12.13 for the white types and from 9.25 to 10.84 for the yellow types of whole maize seeds.

The inadequacy of its proteins for growth, that is, its deficiencies in certain essential amino-acids, has long been realised. These deficiencies were shown to be lysine and tryptophane, and Osborne and Mendel (1914) also pointed out that maize proteins were comparatively low in arginine and histidine. They suggested that an improvement may result in maize feeds by increasing the arginine content. Mitchell and Smuts (1932) improved the biological value of whole maize by the supplementation with lysine and tryptophane, and they concluded from these results that lysine was the primary deficiency.

Various workers have shown that the biological value of maize varies at different levels of protein intake. Thus Mitchell *et al* (1924) found an average biological value of 59.6 for rats fed at 10 per cent. protein level and one of 72.0 at 5 per cent. protein level, indicating a lower biological value when the protein level was raised. Similarly Boas-Fixsen (1932) using only 3 rats reported a biological value of 67 for yellow maize fed at an 8 per cent. protein level and one of 84 at a 5 per cent. protein level. Mitchell and Kick (1935) fed maize to pigs at an 8 per cent. protein level and found an average biological value of 54.

Yet another factor which may contribute to variations in the biological values of maize proteins is suggested by the work of Laporta *et al* (1937) who showed that the proteins of the germ of maize differed from those of the rest of the maize seed, a biological value of 60 being obtained for the whole seed as compared with one of only 50 when the germ was removed. Mitchell and Beadles (1944) recently reported a value of 77.6 for the biological value of the germ of maize.

Marais and Smuts (1940) found a biological value of 76 for a specimen of whole white maize as against one of 67 for a yellow variety. Repetition of the test on a second sample of white maize yielded an almost identical figure for the biological value, viz., 75 (unpublished data). Subsequently the writer determined the biological value of a sample of white maize and of one of the yellow varieties from new consignments and found values which were almost the exact reverse of those reported by Marais and Smuts. These findings led to an extension of the work to a number of varieties of maize which were kindly supplied by the Summer Rainfall Cereal Research Station, Kroonstad, Orange Free State, and by the Potchefstroom Agricultural College Farm, and resulted in the abandonment of an idea which took form at the time of the earlier determinations at this station to the effect that the proteins of white maize are necessarily superior to those of yellow maize.

EXPERIMENTAL.

The biological values of the proteins of thirteen varieties of maize were calculated from data obtained in metabolism studies with male rats of the Wistar strain. The method of experimentation, involving the principle of Mitchell (1924), was that described by Marais and Smuts (1940). Eleven of the samples of maize, with protein contents varying between 10 and 12.2 per cent., were obtained, as previously stated, from Kroonstad and Potchefstroom, the remaining two samples, designated M and N, were taken from supplies available in bulk for general feeding purposes on the station. With the exception of one yellow variety (sample G.) the percentage utilization of whose proteins was confirmed in a second trial, the biological value of the proteins of each variety of maize was determined once only with six rats. The low nitrogen period was conducted, prior to the protein period in the case of samples M and N, but following the protein period in the case of all other samples.

RESULTS.

The percentage composition of the experimental rations are given in Tables 1 and 2, whilst the essential results of the several trials are detailed in Tables 3 and 4, and summarized in Table 5. The procedure for the analysis of variance has been applied to the data. The necessary differences for significance between means were found to be 3.442 at $P = .05$ and 4.578 at $P = .01$. The statistical significance of the differences among the mean biological values given in Table 5 is indicated in Table 6.

It will be noted from a study of Table 5 that the average biological values of the proteins of the yellow varieties of maize are generally higher than those for the white varieties, even if the values obtained for samples M and N by running the low nitrogen period before the protein period, a procedure which may result in unduly high biological values [Mitchell and Beadles (1937)], are discounted. These results reverse previous impressions in regard to the nutritive value of the proteins of the two classes of maize by showing that the white varieties are not necessarily superior to the yellow varieties.

Marais and Smuts (*loc. cit.*) thought that variations in the biological values of the proteins of maize may be due to constitutional differences in the protein moieties. An alternative explanation is suggested by the results of Laporta *et al.* and of Mitchell and Beadles, referred to in the introduction to this paper, to the effect that the proteins of the germ of the maize seed

are better balanced in respect of essential amino acids than are those of the rest of the seed. If this be so, then differences in the biological value of the proteins of whole seed may simply result from quantitative differences in the ratio of embryo to endosperm, inherent in different varieties of maize or caused by varying climatic conditions in the course of the development of the seeds. Direct experimentation on this particular aspect of the problem is indicated.

SUMMARY.

1. The biological values of yellow maize and white maize are given for some eleven varieties grown under South African (summer rainfall) conditions.

2. The results indicate that there exists a significant difference in the nutritive value of the proteins of some of these varieties, but that these differences are not associated with the colour of the maize.

ACKNOWLEDGMENT.

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TABLE 1.
Percentage Composition of the Rations.

Ingredients.	N-Low.	Whole Yellow Maize M.	Whole White Maize N.	Remarks.
Whole Yellow Maize M(Commercial type)	—	80.0	—	1. Whole egg was dried on the steam bath and ether extracted.
Whole White Maize N(Commercial Type)	—	—	83.3	2. Butterfat was prepared from butter, after filtration of the heat-coagulated casein.
Whole Egg.....	3.8	—	—	3. Harris yeast concentrate. Prepared by the Harris Laboratory, Tuckahoe, New York.
Sucrose.....	10.0	5.0	1.7	4. Hubbel Salt Mixture—see Hubbel <i>et al.</i> (1937), <i>J. Nutrition</i> , Vol. 14 p. 273.
Butterfat.....	8.0	8.0	8.0	
Harris Yeast.....	2.0	2.0	2.0	
Cod Liver Oil.....	2.0	2.0	2.0	
Salt mixture.....	2.0	2.0	2.0	
Dextrinized Starch.....	69.2	—	—	
NaCl.....	1.0	1.0	1.0	
Agar.....	2.0	—	—	
Total.....	100.0	100.0	100.0	
Percentage N.....	0.60	1.267	1.290	

TABLE 2.
Percentage Composition of the Rations.

Ingredients.	N.Low.	Whole Yellow Maize. A.	Whole Yellow Maize. B.	Whole Yellow Maize. C.	Whole Yellow Maize. D.	Whole Yellow Maize. E.	Whole Yellow Maize. F.	Whole Yellow Maize. G.	Whole Yellow Maize. H.	Whole White Maize. J.	Whole White Maize. K.	Whole White Maize. L.
A variety "Ekateen"	—	71.0	—	—	—	—	—	—	—	—	—	—
B Variety "Blits"	—	—	64.6	—	—	—	—	—	—	—	—	—
C Variety "Robyn"	—	—	70.0	—	—	—	—	—	—	—	—	—
D Variety "Hotnot"	—	—	—	64.2	—	—	—	—	—	—	—	—
E Variety "Sabara"	—	—	—	—	72.9	—	—	—	—	—	—	—
F Variety "Peruvian"	—	—	—	—	—	73.7	—	—	—	—	—	—
G Commercial Type	—	—	—	—	—	—	77.8	—	—	—	—	—
H Same as G	—	—	—	—	—	—	77.8	—	74.2	—	—	—
I Variety "American Flint"	—	—	—	—	—	—	—	—	—	—	—	—
J Variety "Anveld"	—	—	—	—	—	—	—	—	—	70.7	—	—
K Variety "Hickory King"	—	—	—	—	—	—	—	—	—	—	70.4	—
L Variety "Potchefstroom Pearl"	—	—	—	—	—	—	—	—	—	—	—	69.0
Whole Egg	3.8	—	—	—	—	—	—	—	—	—	—	—
Sucrose	10.0	12.0	18.4	13.0	18.8	10.1	9.3	7.2	7.2	12.3	12.6	14.0
Butterfat	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Harris Yeast	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Cod Liver Oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Hubbel Salt Mixture	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Dextrinized Starch	69.2	—	—	—	—	—	—	—	—	—	—	—
NaCl	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Agar	2.0	2.0	2.0	2.0	2.0	2.0	2.0	—	2.0	2.0	2.0	2.0
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Percentage N	0.60	1.210	1.280	1.295	1.330	1.310	1.375	1.270	1.288	1.220	1.293	1.291

REMARKS.—1. Whole egg was dried on water-bath and ether extracted.
 2. Butterfat was prepared from butter by filtering off the heat-precipitated casein.
 3. Harris Yeast Concentrate—The Harris Laboratory, Tuckahoe, New York.
 4. Hubbel Salt Mixture—Hubbel *et al.* *J. Nutrition* (1937), Vol. 14, p. 273.

TABLE 3.
Nitrogen Metabolism Data—Calculation of the Biological Value.

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N-Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Nett N-Utilization.
							Per Gm. Food.	Per Day.				Per 100 Gm. Weight.	Per Day.							
N-low Period.																				
54	157	159	158	gm. 10.5	gm. —	gm. 25.9	gm. 2.46	gm. —	gm. —	gm. —	gm. 19.1	gm. 12.1	gm. —	gm. —	gm. —	gm. —	—	—	—	—
55	169	162	166	9.0	—	23.8	2.65	—	—	—	17.8	10.7	—	—	—	—	—	—	—	—
56	167	167	167	10.0	—	22.4	2.24	—	—	—	19.6	11.7	—	—	—	—	—	—	—	—
57	161	161	161	9.9	—	17.8	1.80	—	—	—	19.5	12.1	—	—	—	—	—	—	—	—
58	160	166	158	8.8	—	20.9	2.37	—	—	—	19.9	12.6	—	—	—	—	—	—	—	—
59	170	174	172	10.3	—	25.8	2.50	—	—	—	18.1	10.5	—	—	—	—	—	—	—	—
Whole White Maize (N) Period (N = 1.29 per cent.)																				
54	163	164	164	10.5	135.4	24.0	2.46	25.82	-1.82	135.4	73.0	12.1	19.8	53.2	82.2	+38.4	82	100	60.7	60.7
55	152	152	152	9.0	116.0	19.5	2.65	23.88	-4.38	116.0	60.1	10.7	16.2	43.9	72.1	+36.4	83	100	62.2	62.2
56	173	173	173	10.0	129.0	24.0	2.24	22.40	+1.60	127.4	54.9	11.7	20.2	34.7	92.7	+49.1	82	99	72.7	72.0
57	161	165	163	9.9	127.8	23.6	1.80	17.80	+5.80	122.0	59.3	12.1	19.7	39.6	82.4	+44.9	82	96	67.6	65.0
58	159	160	160	9.5	122.5	22.2	2.37	22.60	-0.30	122.5	50.0	12.6	20.2	29.8	92.7	+50.3	84	100	75.7	75.7
59	174	178	176	10.4	134.1	26.0	2.50	26.00	0	134.1	64.9	10.5	18.5	46.4	87.7	+43.2	81	100	65.5	65.5
AVERAGE.....																	82	99	67.4	66.8

TABLE 3—(continued).

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N-Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Net N-Urtilization.
							Per Gm. Food.	Per Day.				Per 100 Gm.	Per Day.							
N-low Period.																				
48	177	180	179	10.7	gm.	mgm.	mgm.	2.28	mgm.	mgm.	23.6	mgm.	13.2	mgm.	mgm.	mgm.	—	—	—	—
49	183	186	185	10.6	—	23.1	2.18	—	—	—	23.0	12.4	—	—	—	—	—	—	—	—
50	164	162	163	8.7	—	20.3	2.34	—	—	—	25.4	15.6	—	—	—	—	—	—	—	—
51	180	177	179	9.7	—	23.3	2.40	—	—	—	24.1	13.5	—	—	—	—	—	—	—	—
52	170	169	170	10.0	—	20.2	2.02	—	—	—	19.5	11.5	—	—	—	—	—	—	—	—
53	164	162	163	9.7	—	21.2	2.19	—	—	—	23.8	14.6	—	—	—	—	—	—	—	—
Whole Yellow Maize (M) Period (N - 1.267 per cent.).																				
48	186	194	190	11.0	139.4	27.4	2.28	25.1	+2.3	137.2	64.0	13.2	25.1	38.9	98.3	+48.0	80	98	71.6	70.2
49	190	198	194	10.5	133.3	26.9	2.18	22.9	+4.0	129.3	60.0	12.4	24.1	35.9	93.4	+46.4	80	97	72.2	70.0
50	169	175	172	9.0	114.0	22.6	2.34	21.1	+1.5	112.5	45.5	15.6	26.8	18.7	93.8	+45.9	80	99	83.5	82.6
51	181	190	186	10.0	126.7	23.7	2.40	24.0	-0.3	126.7	58.8	13.5	25.1	33.7	93.0	+44.2	81	100	73.4	73.4
52	177	182	180	9.9	125.5	24.6	2.02	20.0	+4.6	120.9	53.0	11.5	20.7	32.3	88.6	+47.9	80	96	73.4	70.5
53	169	178	174	10.0	126.7	25.2	2.19	21.9	+3.3	123.4	55.7	14.6	25.4	30.3	93.1	+45.8	79	97	75.5	73.2
AVERAGE.....																	80	98	74.9	73.3

TABLE 4.
Nitrogen Metabolism Data—Calculation of the Biological Value.

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N-Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Net N-Utilization.
							Per Gm. Food.	Per Day.				Per 100 Gm.	Per Day.							
Whole Yellow Maize (A)—“Eksteen”—Period (N = 1.21 per cent.).																				
1	77	77	77	6.0	72.6	14.8	2.03	12.2	+2.6	70.0	38.6	22.6	17.4	21.2	48.8	+19.2	79.6	96.4	69.8	67.2
2	80	78	79	5.9	71.4	12.2	1.63	9.6	+2.6	68.8	38.2	16.6	13.1	25.1	43.7	+21.0	82.8	96.4	63.5	61.3
3	71	71	71	5.5	65.4	10.4	2.00	11.0	-0.6	65.4	33.8	21.2	15.0	18.8	46.6	+21.2	82.5	100	71.2	71.2
4	80	81	81	6.0	72.6	9.8	1.69	10.1	-0.3	72.6	34.9	19.7	15.9	19.0	53.6	+27.9	86.4	100	73.8	73.8
5	95	95	95	7.0	84.7	13.2	2.20	15.4	-2.2	84.7	43.4	21.0	19.0	24.4	60.3	+28.1	84.5	100	71.2	71.2
6	74	77	76	5.9	71.4	11.0	1.93	11.4	-0.4	71.4	35.8	18.7	14.2	21.6	49.8	+24.6	69.8	100	69.8	69.8
														Average.....		83	99	69.9	69.1	
N-low Period.																				
1	76	75	76	6.0	—	12.2	2.03	—	—	—	—	17.2	22.6	—	—	—	—	—	—	—
2	81	82	82	6.0	—	9.8	1.63	—	—	—	—	13.6	16.6	—	—	—	—	—	—	—
3	66	68	67	5.6	—	11.2	2.00	—	—	—	—	14.2	21.2	—	—	—	—	—	—	—
4	77	78	78	6.5	—	11.0	1.69	—	—	—	—	15.4	19.7	—	—	—	—	—	—	—
5	92	93	93	7.0	—	15.4	2.20	—	—	—	—	19.5	21.0	—	—	—	—	—	—	—
6	77	79	78	6.0	—	11.6	1.93	—	—	—	—	14.6	18.7	—	—	—	—	—	—	—

TABLE 4—(continued).

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N-Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Nett N-Utillation.
							Per Gm. Food.	Per Day.				Per 100 Gm.	Per Day.							
Whole Yellow Maize (B)—“Blits”—Period (N=1.28 per cent.).																				
7	77	75	76	6.0	76.8	16.5	1.97	11.8	+4.7	72.1	39.8	21.3	16.2	23.6	48.5	+20.5	78.5	94.0	67.2	63.2
8	75	75	75	5.5	70.4	11.0	2.07	11.4	-0.4	70.4	34.2	18.7	14.0	20.2	50.2	+25.2	84.5	100.0	71.3	71.3
9	76	77	77	5.6	71.7	10.2	2.47	13.8	-3.6	71.7	34.1	19.8	14.3	18.8	52.9	+27.4	85.8	100.0	73.8	73.8
10	81	79	80	6.5	83.2	11.2	1.81	11.8	-0.6	83.2	38.2	17.7	14.2	24.0	59.2	+33.8	86.5	100.0	71.2	71.2
11	75	75	75	6.0	76.8	11.6	1.87	11.2	+0.4	76.4	34.9	18.7	14.2	20.7	55.7	+30.3	85.0	99.5	72.9	72.5
12	88	89	89	7.0	89.5	17.4	1.81	12.6	+4.8	84.7	48.3	18.4	16.4	31.9	52.8	+23.8	80.6	94.7	62.4	59.1
														AVERAGE.....		83	98	69.8	68.5	
N-Low Period.																				
7	73	74	74	6.0	—	11.8	1.97	—	—	—	15.8	21.3	—	—	—	—	—	—	—	—
8	76	75	76	6.0	—	12.4	2.07	—	—	—	14.2	18.7	—	—	—	—	—	—	—	—
9	77	78	78	6.0	—	14.8	2.47	—	—	—	15.5	19.8	—	—	—	—	—	—	—	—
10	77	82	80	6.5	—	11.8	1.81	—	—	—	14.2	17.7	—	—	—	—	—	—	—	—
11	75	76	76	6.0	—	11.2	1.87	—	—	—	14.2	18.7	—	—	—	—	—	—	—	—
12	86	87	87	6.5	—	11.8	1.81	—	—	—	16.0	18.4	—	—	—	—	—	—	—	—

TABLE 4—(continued).

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N-Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Nett N-Utilization.
							Per Gm. Food.	Per Day.				Per 100 Gm.	Per Day.							
Whole Yellow Maize (C)—"Robyn"—Period (N=1.295 per cent.).																				
13	102	107	105	7.0	90.6	12.8	1.86	13.0	-0.2	90.6	50.3	13.1	13.8	36.5	54.1	+27.5	84.8	100.0	59.7	59.7
14	92	96	94	7.0	90.6	16.8	1.81	12.6	+4.2	86.4	49.6	15.9	15.0	34.6	51.8	+24.2	81.5	95.4	60.0	57.2
15	99	106	103	7.0	90.6	12.6	2.14	15.0	-2.4	90.6	48.0	12.3	12.7	35.3	55.3	+29.3	86.0	100.0	61.0	61.0
16	99	102	101	7.0	90.6	13.0	1.57	11.0	+2.0	88.6	48.7	15.3	15.5	33.2	55.4	+26.9	83.5	95.6	62.6	59.9
17	87	93	90	6.5	84.2	13.6	1.90	12.4	+1.2	83.0	46.5	12.8	11.5	35.0	48.0	+24.1	84.0	98.6	58.0	57.2
18	104	108	106	7.0	90.6	12.0	1.97	13.8	-1.8	90.6	48.0	12.2	12.9	35.1	55.5	+30.6	86.8	100.0	61.2	61.2
															AVERAGE.....		84.4	98.3	60.4	59.4
N-low Period.																				
13	106	105	106	7.0	—	13.0	1.86	—	—	—	13.85	13.1	—	—	—	—	—	—	—	—
14	95	99	97	6.5	—	11.8	1.81	—	—	—	15.4	15.9	—	—	—	—	—	—	—	—
15	103	103	103	7.0	—	15.0	2.14	—	—	—	12.6	12.3	—	—	—	—	—	—	—	—
16	101	104	103	7.0	—	10.96	1.57	—	—	—	15.8	15.3	—	—	—	—	—	—	—	—
17	92	91	92	6.0	—	11.4	1.90	—	—	—	11.8	12.8	—	—	—	—	—	—	—	—
18	105	108	107	7.0	—	13.8	1.97	—	—	—	13.0	12.2	—	—	—	—	—	—	—	—

TABLE 4—(continued).

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N-Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Nett N-Urtilization.
							Per Gm. Food.	Per Day.				Per 100 Gm.	Per Day.							
Whole Yellow Maize (D)—“Hotnot”—Period (N = 1.33 per cent.).																				
19	94	99	97	7.0	93.1	15.8	1.83	12.8	+3.0	90.1	55.6	12.9	12.5	43.1	57.0	+21.7	83.0	97.0	63.3	61.4
20	107	111	109	7.0	93.1	18.3	2.28	16.0	+2.3	90.3	52.0	15.9	17.3	34.7	56.1	+22.8	80.4	97.6	61.8	60.2
21	107	110	109	7.0	93.1	16.4	1.71	12.0	+4.4	88.7	53.6	15.6	17.0	36.6	52.1	+23.1	82.4	95.2	59.0	56.2
22	101	108	105	7.0	93.1	18.1	1.71	12.0	+6.1	87.0	50.0	15.4	16.2	33.8	53.2	+25.0	80.6	93.4	61.1	57.0
23	92	98	95	7.0	93.1	15.8	2.03	14.2	+1.6	91.5	46.0	13.6	12.9	33.1	58.4	+21.3	83.0	98.2	53.8	62.6
24	104	106	105	7.0	93.1	15.8	1.63	11.4	+4.4	88.7	56.0	18.7	19.6	36.4	52.3	+21.3	83.0	95.2	59.0	56.2
AVERAGE.....														82.1	96.1	61.3	58.9			
N-low Period.																				
19	96	99	98	7.0	—	12.8	1.83	—	—	—	—	12.6	12.9	—	—	—	—	—	—	—
20	108	106	107	6.5	—	14.8	2.28	—	—	—	—	17.0	15.9	—	—	—	—	—	—	—
21	107	110	109	7.0	—	12.0	1.71	—	—	—	—	17.0	15.6	—	—	—	—	—	—	—
22	102	97	100	6.0	—	10.17	1.71	—	—	—	—	15.43	15.4	—	—	—	—	—	—	—
23	93	93	93	6.5	—	13.2	2.03	—	—	—	—	12.6	13.6	—	—	—	—	—	—	—
24	106	103	104	7.0	—	11.36	1.63	—	—	—	—	19.5	18.7	—	—	—	—	—	—	—

TABLE 4—(continued).

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N-Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Net N-Utilization.
							Per Gm. Food.	Per Day.				Per 100 Gm. Weight.	Per Day.							
25	154	157	156	8.4	110.0	18.5	2.40	20.2	-1.7	110.0	66.1	11.4	17.8	48.3	61.7	+26.4	83	100	56.1	56.1
26	146	149	148	7.0	91.7	19.3	2.37	15.9	+3.4	88.3	52.0	13.2	19.5	32.5	55.8	+20.4	79	96	63.2	60.7
27	152	152	152	7.1	93.0	17.1	2.09	14.55	+2.25	90.75	57.3	13.4	20.4	36.9	53.8	+18.6	82	98	59.4	58.2
28	164	169	167	8.4	110.0	18.9	2.09	17.55	+1.35	108.6	65.0	12.7	21.4	43.6	65.0	+16.1	83	99	59.9	59.3
29	161	161	156	8.4	110.0	19.7	2.31	19.4	+0.3	109.7	61.3	12.4	19.3	42.0	67.7	+29.0	82	100	61.6	61.6
30	154	155	155	8.4	110.0	18.5	2.09	17.6	+0.9	109.1	65.4	11.5	17.8	47.6	61.5	+26.1	83	99	56.4	55.8
AVERAGE.....																	82	98	59.3	58.6

Whole Yellow Maize (E)—"Sahara"—Period (N-1.31 per cent.).

N-low Period.

25	150	146	148	8.0	—	19.15	2.40	—	—	—	16.9	11.4	—	—	—	—	—	—	—	—
26	142	142	142	7.5	—	17.75	2.37	—	—	—	18.75	13.2	—	—	—	—	—	—	—	—
27	144	144	144	8.5	—	17.75	2.09	—	—	—	19.4	13.4	—	—	—	—	—	—	—	—
28	153	158	156	8.5	—	17.75	2.09	—	—	—	19.8	12.7	—	—	—	—	—	—	—	—
29	151	151	151	8.0	—	18.5	2.31	—	—	—	18.75	12.4	—	—	—	—	—	—	—	—
30	142	145	144	7.5	—	15.7	2.09	—	—	—	16.5	11.5	—	—	—	—	—	—	—	—

TABLE 4—(continued).

[illegible]

Y-low Period.

[illegible]

TABLE 4—(continued).

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N-Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Net N-Utilization.
							Per Gm. Food.	Per Day.				Per 100 Gm.	Per Day.							
Whole Yellow Maize (G) Period (N = 1.288 per cent.).																				
36	91	100	96	10.9	140.4	26.1	2.35	25.7	+0.4	140.0	71.0	18.3	17.6	53.4	86.6	+43.3	81	97	61.9	60.0
37	95	103	99	10.9	140.4	26.3	2.57	28.0	-1.7	149.4	72.6	17.7	17.5	55.1	85.3	+41.5	81	100	60.8	60.8
38	93	103	98	9.3	119.6	20.6	2.62	24.3	-3.7	119.6	70.4	18.0	16.8	53.6	66.0	+28.6	83	100	55.2	55.2
39	100	106	103	9.7	124.9	22.2	2.52	24.4	-2.2	124.9	68.7	17.3	17.8	50.9	74.0	+34.0	82	100	59.2	59.2
40	87	97	92	10.0	128.8	21.4	2.53	25.3	-3.9	128.8	72.0	18.3	16.9	55.1	73.7	+35.4	83	100	57.2	57.2
41	86	91	89	9.8	126.2	23.5	2.74	26.8	-3.3	126.2	68.9	16.7	14.9	54.0	72.2	+33.8	81	100	57.2	57.2
AVERAGE.....														82	99	58.6	58.3			
N-lour Period.																				
36	101	107	104	9.9	—	23.3	2.35	—	—	—	19.0	18.3	—	—	—	—	—	—	—	—
37	105	112	109	9.8	—	25.3	2.57	—	—	—	19.2	17.7	—	—	—	—	—	—	—	—
38	106	110	108	7.4	—	19.4	2.62	—	—	—	19.4	18.0	—	—	—	—	—	—	—	—
39	108	110	108	7.7	—	19.4	2.52	—	—	—	18.7	17.3	—	—	—	—	—	—	—	—
40	95	100	98	7.5	—	19.0	2.53	—	—	—	17.9	18.3	—	—	—	—	—	—	—	—
41	93	96	95	7.3	—	20.0	2.74	—	—	—	15.9	16.7	—	—	—	—	—	—	—	—

TABLE 4—(continued).

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Per Day.	Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Per Day.	Food N in Urine.	Retained N.	N-Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Nett N-Fertilization.
							Per Gm. Food.	N.					Per 100 Gm.	Weight.								
42	140	141	141	9.5	120.6	21.85	2.40	22.8	22.5	-0.95	120.6	70.8	11.4	16.1	54.7	65.9	mgm.	+27.9	81	100	64.5	54.5
43	140	147	144	9.5	120.6	21.05	2.37	22.5	22.5	-1.45	120.6	63.0	13.2	19.0	44.0	76.6	mgm.	+36.5	82	100	63.5	63.5
44	135	140	138	9.5	120.6	18.56	2.09	19.9	19.9	-1.34	120.6	70.0	13.4	18.5	51.5	69.1	mgm.	-32.0	85	100	57.3	47.3
45	138	147	143	9.7	123.0	20.4	2.09	20.3	20.3	+0.1	122.9	68.8	12.7	18.1	50.7	72.2	mgm.	+33.8	83	100	58.7	58.7
46	144	149	149	10.1	128.1	21.85	2.31	23.4	23.4	-1.65	128.1	70.8	12.4	18.5	52.3	75.8	mgm.	+35.4	83	100	59.2	59.2
47	130	138	134	9.1	115.5	22.5	2.09	19.0	19.0	+3.5	112.0	63.0	11.5	15.4	47.6	64.4	mgm.	+30.0	80	98	57.6	56.4
AVERAGE.....																			82	100	58.5	58.3

Y-low Period.

[illegible]

TABLE 4—(continued).

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N-Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Net N-Utilization.
							Per Gm. Food.	Per Day.				Per 100 Gm.	Per Day.							
Whole White Maize (H)—“American Flint”—Period (N = 1.270 per cent.).																				
78	90	92	91	7.0	88.9	16.0	2.00	14.0	+2.0	86.9	55.3	24.5	22.3	34.0	52.9	+16.6	82.0	96.6	60.9	89.8
79	104	105	105	8.0	101.6	18.7	2.36	18.9	-0.2	101.6	63.4	21.9	23.0	40.4	61.2	+19.5	81.0	100.0	60.3	60.3
80	94	99	97	7.5	95.2	16.8	2.15	16.1	+0.7	94.5	53.2	20.2	19.6	33.6	60.9	+25.2	82.3	99.3	64.4	64.0
81	112	116	114	9.0	114.4	20.4	2.36	21.2	-0.8	112.2	63.1	19.0	21.7	41.4	70.8	+30.9	82.2	100.0	63.1	63.1
82	103	107	104	8.0	101.6	20.5	2.31	18.5	+2.0	99.6	64.3	18.0	18.7	45.6	54.0	+16.8	80.0	98.0	54.3	53.2
83	107	111	109	8.5	108	21.7	2.44	20.8	+0.9	107.1	64.0	18.7	20.4	43.6	63.5	+22.3	80.0	99.1	59.3	58.7
AVERAGE.....														81	99	60.4	59.7			
N-lour Period.																				
78	91	93	92	7.5	—	14.95	2.00	—	—	—	22.6	24.5	—	—	—	—	—	—	—	—
79	102	105	104	8.5	—	20.1	2.36	—	—	—	22.8	21.9	—	—	—	—	—	—	—	—
80	97	99	98	7.5	—	16.1	2.15	—	—	—	19.8	20.2	—	—	—	—	—	—	—	—
81	155	115	115	8.0	—	18.9	2.36	—	—	—	21.9	19.0	—	—	—	—	—	—	—	—
82	106	110	108	8.0	—	18.5	2.31	—	—	—	19.4	18.0	—	—	—	—	—	—	—	—
83	109	110	110	8.0	—	19.5	2.44	—	—	—	20.6	18.7	—	—	—	—	—	—	—	—

TABLE 4—(continued).

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N-Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Nett N-Utization.
							Per Gm. Food.	Per Day.				Per 100 Gm. Wt.	Per Day.							
Whole White Maize (J)—“Anveld”—Period (N=1.22 per cent.).																				
84	84	87	86	7.0	85.4	14.3	2.19	15.3	-1.0	85.4	56.8	21.6	18.6	38.2	47.2	+14.3	83.3	100	55.3	55.3
85	86	90	88	7.0	85.4	13.2	2.67	18.7	-6.6	85.4	58.8	20.2	17.8	41.0	44.4	+13.4	84.6	100	52.1	52.1
86	85	90	88	7.0	85.4	13.8	2.64	18.5	-5.5	85.5	56.3	20.4	18.1	38.2	47.2	+15.3	83.8	100	55.3	55.3
87	87	91	89	7.0	85.4	14.7	2.25	16.8	-1.1	85.4	56.9	23.4	20.8	36.1	49.3	+13.8	82.8	100	57.7	57.7
88	72	75	74	6.0	73.2	11.3	2.33	14.0	-2.7	73.2	47.1	22.8	16.9	30.2	43.0	+14.8	86.0	100	58.7	58.7
89	65	69	67	6.0	73.2	14.9	2.37	14.2	+0.7	72.5	48.2	22.4	15.0	33.2	39.3	+10.1	79.8	99	54.2	53.7
AVERAGE.....															83	100	55.5	55.5		
N-low Period.																				
84	88	91	90	7.0	—	15.3	2.19	—	—	—	19.4	21.6	—	—	—	—	—	—	—	—
85	90	92	91	7.0	—	18.7	2.67	—	—	—	18.3	20.2	—	—	—	—	—	—	—	—
86	94	96	95	7.0	—	18.5	2.64	—	—	—	19.4	20.4	—	—	—	—	—	—	—	—
87	90	91	91	7.5	—	16.9	2.25	—	—	—	21.3	23.4	—	—	—	—	—	—	—	—
88	74	74	74	6.0	—	14.0	2.33	—	—	—	16.9	22.8	—	—	—	—	—	—	—	—
89	67	69	68	6.5	—	15.1	2.37	—	—	—	15.2	22.4	—	—	—	—	—	—	—	—

TABLE 4—(continued).

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N-Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Net N-Utillation.
							Per Gm. Food.	Per Day.				Per 100 Gm. Weight.	Per Day.							
<i>Whole White Maize (K)—"Hickory King"—Period (N = 1.293 per cent.).</i>																				
90	108	110	109	7.5	97.0	22.2	2.20	21.3	+0.9	96.1	62.2	23.7	25.8	36.4	59.7	+12.6	77.1	99.0	62.1	61.4
91	115	120	118	8.5	110.0	21.8	2.24	19.1	+2.7	107.3	72.8	22.8	26.9	45.9	61.4	+15.4	80.2	98.6	57.3	56.5
92	112	115	114	8.5	110.0	20.0	2.33	19.8	+0.2	109.8	70.0	19.7	22.5	47.5	62.3	+20.0	81.9	99.8	56.8	56.7
93	97	99	98	7.0	90.6	17.2	2.33	16.3	+0.9	89.7	54.1	21.8	21.4	32.7	57.0	+19.3	81.0	99.0	63.6	63.0
94	107	113	110	8.0	103.4	16.8	2.20	17.6	-0.8	103.4	58.9	21.7	23.9	35.0	68.4	+27.7	83.8	100.0	66.2	66.2
95	99	100	100	7.0	90.6	17.6	2.23	15.6	+2.0	88.6	55.3	20.1	20.1	35.2	53.4	+17.7	80.6	98.4	60.3	58.3
														AVERAGE.....		81	99	61.0	60.5	

N-low Period.

90	108	112	110	7.5	—	16.8	2.20	—	—	—	26.0	23.7	—	—	—	—	—	—	—	—
91	118	123	121	8.0	—	17.9	2.24	—	—	—	27.6	22.8	—	—	—	—	—	—	—	—
92	120	121	121	7.5	—	17.5	2.33	—	—	—	23.8	19.7	—	—	—	—	—	—	—	—
93	100	102	101	6.3	—	14.7	2.33	—	—	—	22.0	21.8	—	—	—	—	—	—	—	—
94	109	114	112	7.0	—	15.4	2.20	—	—	—	24.3	21.7	—	—	—	—	—	—	—	—
95	100	107	104	7.0	—	15.6	2.23	—	—	—	20.9	20.1	—	—	—	—	—	—	—	—

TABLE 4—(continued).

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N-Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Net N-Urtilization.
							Per Gm. Food.	Per Day.				Per 100 Gm.	Per Day.							
Whole White Maize (L)—“Potchefstroom Pearl”—Period (N = 1.291 per cent.).																				
96	128	132	130	9.0	116.0	20.0	2.17	19.5	+0.5	115.5	72.0	17.1	22.2	49.8	65.7	+24.0	82.7	99.6	57.0	56.8
97	117	122	120	8.5	110.0	20.4	2.29	19.5	+0.9	109.1	72.0	17.8	21.4	50.6	58.5	+17.6	81.4	99.2	53.6	53.2
98	125	132	128	9.0	116.0	22.3	2.20	19.8	+2.5	113.5	73.2	18.8	24.1	49.1	64.4	+20.5	80.8	98.0	56.8	56.6
99	130	137	134	9.5	122.5	22.1	2.41	22.9	-0.8	122.5	78.4	18.5	24.7	53.7	68.8	+22.0	82.2	100.0	56.2	56.2
100	131	140	136	10.0	129.1	24.2	2.19	21.9	+2.3	126.8	83.5	19.2	26.1	57.4	59.4	+21.4	81.4	98.2	54.9	54.0
101	120	124	122	8.5	110.0	17.0	2.14	21.4	-4.4	110.0	70.6	19.5	23.8	46.8	63.2	+22.4	84.6	100.0	57.4	57.4
														AVERAGE.....		82	99	56.0	55.7	
N-low Period.																				
96	133	140	137	9.0	—	19.5	2.17	—	—	—	—	23.4	17.1	—	—	—	—	—	—	—
97	124	134	129	8.5	—	19.5	2.29	—	—	—	—	23.0	17.8	—	—	—	—	—	—	—
98	132	140	136	9.0	—	19.8	2.10	—	—	—	—	25.6	18.8	—	—	—	—	—	—	—
99	133	141	137	9.0	—	21.7	2.41	—	—	—	—	25.3	18.5	—	—	—	—	—	—	—
100	138	146	142	9.0	—	19.7	2.19	—	—	—	—	27.3	19.2	—	—	—	—	—	—	—
101	127	135	131	8.5	—	18.2	2.14	—	—	—	—	25.5	19.5	—	—	—	—	—	—	—

TABLE 5.

Percentage Utilization of the Proteins of some South African Varieties of Maize at about 8 Percentage Protein Level.

Variety.	Apparent Digestibility. (Average).	True Digestibility. (Average).	Biological Value. (Average).	Nett Utilization of Nitrogen (Average).
A. Eksteen (yellow).....	83	99	69.9 ± 1.409	69.1
B. Blits (yellow).....	83	98	69.8 ± 1.745	68.5
C. Robyn (yellow).....	84	98	60.4 ± 1.004	59.4
D. Hotnot (yellow).....	82	96	61.3 ± 0.839	58.9
E. Sahara (yellow).....	82	98	59.3 ± 1.146	58.6
F. Peruvian (yellow).....	86	99	58.6 ± 1.338	58.2
G. Commercial (yellow).....	82	99	58.6 ± 1.016	58.3
G1. Commercial (yellow).....	82	100	58.5 ± 1.208	58.3
H. American Flint (white).....	81	99	60.4 ± 1.437	59.7
J. Anveld (white).....	83	100	55.5 ± 0.973	55.5
K. Hickory King (white).....	81	99	61.0 ± 1.472	60.5
L. Potchefstroom Pearl (white).....	82	99	56.0 ± 0.594	55.7
M. Unknown (yellow).....	80	98	74.9 ± 1.798	73.3
N. Unknown (white).....	82	99	67.4 ± 2.398	66.8

TABLE 6.
Statistical Analysis of Results.

Maize Varieties.	A.	B.	C.	D.	E.	F.	G.	H.	J.	K.	L.	M.	N.
A. Eksteen.....	—	0	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	0
B. Blits.....	0	—	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	0
C. Robyn.....	XX	XX	—	0	0	0	0	0	XX	0	X	XX	XX
D. Hotnot.....	XX	XX	0	—	0	0	0	0	XX	0	X	XX	XX
E. Sahara.....	XX	XX	0	0	—	0	0	0	X	0	0	XX	XX
F. Peruvian.....	XX	XX	0	0	0	—	0	0	0	0	0	XX	XX
G. Commercial.....	XX	XX	0	0	0	0	—	0	0	0	0	XX	XX
H. American Flint.....	XX	XX	0	0	0	0	0	—	XX	0	X	XX	XX
J. Anveld.....	XX	XX	X	X	X	0	0	X	—	X	0	XX	XX
K. Hickory King.....	XX	XX	0	0	0	0	0	0	XX	—	XX	XX	XX
L. Potchefstroom Pearl....	XX	XX	X	XX	0	0	0	X	0	XX	—	XX	XX
M. Unknown.....	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	—	XX
N. Unknown.....	0	0	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	—

0 = No significant differences.

X = Significant difference at $P = .05$.

XX = Significant difference at $P = .01$.

The Familial Incidence of Spontaneous *Osteopetrosis gallinarum*.

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Onderstepoort.

OSTEOPETROSIS is a rare disease of the domestic fowl and we agree with the statement of Jungherr and Landauer that one case can be expected in about every two thousand birds sent for examination by farmers. This is all the more remarkable when it is realised how comparatively simple it is to detect the abnormality, for the long bones show the diaphyses increased in their overall diameter and the osseous tissue is very much like marble. The shanks are so often involved that the diagnosis becomes even easier. Some cases are revealed only when the hardness of the bones is noticed at table.

In 1932 Hutt found two of nine fowls from the same dam affected, and very properly considered that the condition might be a genetic recessive character. During the same year, Jungherr and Landauer found a case in the flocks kept at the Storrs Agricultural Experiment Station, and in 1933 there were 30 cases, and in 1934 the number rose to 40. From 1935 onwards the cases rapidly became fewer. One might have expected support to have been forthcoming for the belief of Hutt, but Jungherr and Landauer stated, "A careful analysis of the breeding records did not suggest either a familial or hereditary tendency in the occurrence of the condition".

In 1938 the first case of osteopetrosis was diagnosed in the pedigreed flock of Single Comb White Leghorns at Onderstepoort. It was decided to pay particular attention to the disease and see if it would not tend to stigmatize certain families more than others. Of the fowls hatched in 1937, two developed osteopetrosis; the figure for 1938 was one, for 1939 five, for 1940 twenty-three, for 1941 six, and for 1942 nought. A peak was thus reached in 1940 resembling that observed by Jungherr and Landauer in 1934.

The most striking fact about the disease at Onderstepoort has been its frequent development in the members of certain families, and the main purpose of this article is to present evidence in support of Hutt's suggestion. It is believed that at least the *susceptibility* of the fowl to osteopetrosis is a recessive character, possibly a simple recessive character. In addition to this, it was concluded that the incidence of neurolymphomatosis, carcinosis and leucosis in birds with osteopetrosis is not significantly higher than in the flocks as a whole.

REVIEW OF THE VETERINARY LITERATURE.

In 1924 Ball and Auger described the disease in a hen in France. The periosteum was firmly attached to the affected bones. The viscera were normal, except for a tubercle in the liver. From this article it would appear that Besnoit and Robin encountered the disease in 1922 in a cock, that was also tubercular.

Pugh studied what was apparently the same disease in England and submitted his findings in 1927. A poultry farmer examined 30,000 fowls of both sexes over a period of years and found 44 cases, all in cockerels. Cross-bred and pure-bred birds were equally susceptible. The fowls were raised on open range, and symptoms were usually first observed at the age of six to seven months. The long bones of the legs and wings often become enormously increased in diameter, without any definite signs of ill health appearing. Lameness or a stilted gait followed when the metatarsal bones became so enlarged that the flexor tendons could not function freely. In very severe cases, the cockerel could not put its digits down flat when standing on a smooth surface. The joints were neither enlarged nor tender, and could be flexed easily without pain. The lesions were usually, but not always, developed to the same degree on both sides of the body. The tibiae and metatarsi were generally involved, the bones being dense and heavy. The periosteum over the affected bone was invariably thickened and sometimes difficult to remove. The humerus, and then the ulna were the wingbones chiefly affected. The space between the radius and the ulna was sometimes obliterated. The scapulae and coracoids were frequently thickened and shortened. The clavicles were almost invariably smaller than normal. The cranial portion of the sternal crest might be thickened; the same was true of the ilium anterior to the acetabulum. The head, beak, vertebrae and digits were unchanged. The thyroids were occasionally enlarged. The testes were reduced in size by three-quarters. The joints and articular cartilages were never affected. Growth and sexual development were retarded. The disease sometimes became arrested spontaneously after several months and, after "recovery", the cockerel could mature sexually and become fertile. Our hen H. 8330 is an excellent example of sexual maturity being deferred until the age of over eighteen months. Finally, Pugh found no evidence of leucaemia in seven cases that were very thoroughly examined. The most striking feature of Pugh's observations was the fact that only male birds were affected. It is now conceded that both sexes are equally liable to show symptoms.

In 1929 Veenendaal referred very briefly to the disease, but added nothing to the existing knowledge.

Carpentier in 1931 described the affliction in a cockerel of three months. The diagnosis was made after the bird had been cooked.

Bayon mentioned, in 1934, that he had encountered two unrelated cases as a result of performing 1,000 autopsies. One was a cross-bred cockerel. Both branches of the wish bone or furcula were very thickened in the one bird.

In 1935 Patay saw a few cases on a large poultry farm in France, and the usual bones were involved and general development was impaired. He asserted that the parathyroids were hypertrophied.

Brochet published two short articles in 1935 on the disease in a hen, and one was illustrated by good photographs. The tibia was affected mostly,

and the diaphysis was extremely thickened. The clavicle was smaller and thinner than usual. The head, beak, vertebrae, pelvis and nails were normal.

Osteopetrosis, complicated with spirochaetosis, was diagnosed in a White Leghorn hen and cock by Venkataraman in India in 1936. The two birds belonged to the same owner.

The above articles served to draw attention to this typical, if rare, disease of fowls, but threw no light on its causation. It was left to Jungherr and Landauer to publish the first experimental work. In 1935 Jungherr described the endemic at Storrs Agricultural Experiment Station. Various breeds and both sexes were involved, especially males of the Frizzle variety. The disease often manifested itself at the age of six weeks, and was chronic in nature and seldom fatal. It was characterised by the development of large irregular swellings of the leg and wing bones, which were hard. At autopsy, the internal organs were apparently normal and the osseous changes were limited to the long bones. Jungherr also noted what we have repeatedly observed—in the acute or florid stage, the temperature over the surface of the affected bone is perceptibly increased.

In transmission experiments with bacteriologically sterile blood or bone marrow from these florid cases, Jungherr produced four typical examples of osteopetrosis in twenty-two inoculated chicks. Eleven of the remaining eighteen chicks subsequently died or were killed in a debilitated condition and, at autopsy, two showed anaemia, two haemocytoblastomatosis, one lymphoid leucosis and four had gross lesions of neurolymphomatosis. These findings prompted Jungherr to regard osteopetrosis as another manifestation of the leucosis complex.

In 1938, Jungherr and Landauer reported at length on their investigations and suggested the name, *Osteopetrosis gallinarum*, owing to the fact that the disease so closely resembled the condition in man. In 1933 they raised 2,496 fowls and nineteen Creeper, seven Rumpless and four Frizzle subjects were affected. In 1934 they raised 2,035 birds and one Creeper, eighteen Rumpless, eighteen Frizzle and three Cornish fowls showed symptoms. The poultry were fed a proper commercial ration and were kept under semi-range conditions in almost complete quarantine. Both males and females were affected, and although the earliest cases were recognizable at six weeks, some diseased birds lived up to the age of two to three years.

Jungherr and Landauer also diagnosed osteopetrosis in one White Leghorn, two Barred Rocks and two Rhode Island Reds sent for examination by farmers, thus helping to emphasize that all breeds are apparently susceptible.

The following abstracts are taken from the descriptions of the gross pathology of the disease given by Jungherr and Landauer:—

“As the metatarsus, the unfeathered part of the leg, is almost invariably affected the disease is often recognised on clinical examination. In the beginning stages the metatarsus may show a definite convexity of the anterior surface, irregular lumps in the proximal metaphyseal region or thickening of the diaphysis; the affected areas exhibit an increased surface temperature, and are firm and insensitive to the touch. In distinction from perosis, the axis of the extremities remains unaffected. Cases of long standing fail to show the local hyperpyrexia, but are characterised by enormous deformities.

"The gross pathological alterations of the skeleton are fairly consistent in the parts affected, but vary in appearance according to the duration of the condition. The metatarsus, tibia, fibula, femur, humerus, ulna, radius, metacarpus, coracoid, clavicle and sternum may be involved, in a falling order of frequency. The ischium has shown suggestive changes in one instance, while the vertebrae, phalanges and skull bones failed to exhibit either gross or microscopic lesions. The affection is ordinarily bilateral. The integument of the affected bones appears normal; the periosteal cover is usually somewhat more easily detached than in healthy bone. The articular contours of the bones appear to be unaffected. The diaphysis and metaphysis show thickening in various degrees and either a smooth tapering surface or irregular rough excrescences with porous alterations. Even bones in the early stages of the disease offer more resistance to fracture than normal ones, although the hypertrophied areas can be incised to some degree.

"Differences between the florid and arrested stages of the disease are especially pronounced in cross sections. Young lesions are characterised by increased density and enormous hypertrophy of the spongiosa, at the expense of both the marrow cavity and the external outline. Old arrested lesions are extremely hard, and consist of the spongiosa and compacta and an eccentric narrowed medullary cavity. Transitional stages between these extremes are seen. Severe encroachment upon the marrow cavity is pronounced, especially in the diaphyseal region, and seems to lead to complete obliteration in certain areas; the remaining bone marrow of the long bones has often a currant-jelly-like hyperplastic appearance. Variations in the pathologic expression of the disease, progressing from the spongy state toward extensive petrification, can be seen in the same bird. Old lesions tend to occur in the distal, young lesions in the proximal leg bones, or in the wings, and thereby suggest, in line with the clinical course of the disease, the possibility of an ascending development of the affection. A secondary widening of the marrow cavity in the old solid bones may represent an attempt at re-establishing the physiologic balance of the haematopoietic tissues.

"Gross changes in other than the skeletal tissues may be entirely lacking; this holds true especially for birds sacrificed for examination during the initial stages of the disease, or for cases which are discovered on dressing for food purposes. Cases of fairly long standing and especially those which succumb to the malady are apt to show greyish enlargement of the parenchymatous organs, particularly the spleen. In the majority of cases the parathyroids appeared normal."

It was not until the injection of blood or bone marrow from clinically florid cases (with hyperpyretic shanks) was resorted to, that Jungherr and Landauer obtained evidence of transmissibility. The inoculum consisted of fresh, whole heparinized blood or a 10 per cent. suspension of bone marrow in Locke's solution. For preservation, these materials were desiccated over non-fuming concentrated sulphuric acid in a high vacuum, stored in the refrigerator and again suspended in Locke's solution before use. The inoculum never exceeded 0.5 c.c. For the experiments, they injected White Leghorn chicks, less than a week old, that were obtained from a commercial hatchery. Uninoculated controls of the same age were kept in separate batteries and none developed the condition. Transmission of osteopetrosis

was apparently successful with material from two donors from the endemic outbreak, and one strain was carried through four passages. The osseous changes were usually seen after an incubation period of three to five months. The worst bone lesions produced experimentally were never as severe as many found occurring naturally. Microscopically, the experimental cases exhibited well developed lesions, transitional between the florid and arrested stages and resembling in all particulars the pathognomonic alterations of spontaneous osteopetrosis.

The disease was produced when the inoculum was given intracardially, intraperitoneally, intravenously, intracranially and intramuscularly. The evidence pointed to an ultramicroscopic transmissible agent as the aetiological factor, which could withstand desiccation for periods of up to 105 days. The agent appeared to be present in the blood, bone marrow and lymphomata found in some of the cases, but was absent from the diseased bone tissue itself. The aetiological factor could be demonstrated only in active cases, which were characterised by the increased surface temperature already described.

Actually, Jungherr and Landauer injected 61 baby chicks in the course of four passages, and produced six gross lesion cases of osteopetrosis and six gross lesion cases associated with lymphomatosis and twenty-three cases of lymphomatosis. Thus, about 20 per cent. of the experimental chicks showed osseous changes, about 400 times the normal figure, and the results must accordingly be regarded as highly significant. It is interesting to note that no cases developed in the third passage, where the agent presumably remained dormant.

Jungherr and Landauer were impressed by the frequency with which lymphomatosis and neurolymphomatosis occurred in their injected chicks, and this matter will be returned to in due course.

Besides describing the symptoms, gross pathology and histopathology of osteopetrosis, Jungherr and Landauer made an outstanding contribution to the true appreciation of the nature of the disease, by demonstrating the significance of a transmissible agent. The fact that less than 20 per cent. of the injected chicks developed symptoms only tends to show that fowls generally possess a marked natural resistance to the disease. We believe that this natural resistance to the spontaneous, if not to the experimental disease, is an expression of an inherited character.

THE ONDERSTEEPOORT FLOCK OF WHITE LEGHORNS.

This flock is maintained with one aim in view; we desire to breed a strain of fowls highly resistant to neurolymphomatosis and the various neoplasms to which they are liable. Table 1 shows how the mortality from these causes has been reduced by five-sixths, but whether this achievement has been due entirely to a rigid selection of the breeding birds is at present impossible to say. We do not breed from females in their pullet year, and more and more do we insist on the males being in their second season. There are two reasons for this. Firstly, experience has shown that most birds die of malignant conditions and neurolymphomatosis before the age of twenty-one months, the time when they enter the breeding pens. Secondly, little can be known about the egg production of a hen and her sisters, if they are mated when about ten months old.

To gain a place in a breeding pen, a Leghorn hen must lay about 225 eggs in her pullet year, and the eggs must average 2 oz. and be of good shape and have firm shells. The hen must conform to the accepted standard of perfection for the breed, and side sprigs and stubs and crooked keels and spurs must be absent. Her body weight must be about four pounds. The eyes must be a rich bay colour. Broodiness is a disqualification. Early maturing birds, that lay throughout the winter and well into the following autumn, are particularly desired. Potential breeders must come from families where deaths are rare, especially from neoplastic diseases. Preference is given to moderately heavy layers from large families of all-round excellence, rather than to outstanding survivors of families where most have died of lymphoid leucosis, for instance. Cocks are chosen if their sisters have done well, and if they themselves are reasonably fine specimens of the breed. These exacting qualifications are indispensable, if the standard of the poultry is to be raised.

In assembling the breeding pens, it has been our custom to put closely related hens together.

These facts are mentioned for two reasons. Of the 643 hens and 55 cocks used in the breeding pens from 1937 to 1941 only one, a cock, had a brother or a sister that suffered from osteopetrosis, and this cock incidentally sired no cases. The brothers and sisters of cases simply failed to meet the requirements of breeding stock. Then all the sisters, except two, of males and females producing cases were themselves mated to cocks that sired cases. The significance of these two points, fortunate if unremediated, will be appreciated later, for we rely heavily on them in concluding that the susceptibility to osteopetrosis may possibly be inherited as a simple recessive.

THE ONDERSTEEPOORT CASES.

We have already described and indicated in Tables 2 and 3 how affected birds were found among those hatched from 1937 to 1941, with 1940 easily the peak years.

Table 4 gives full details of the diseased fowls, and various deductions may be made. Osteopetrosis seems to occur as readily in one sex as in the other, bearing in mind that fewer males than females are retained. Egg production, if any are laid at all, is very poor; but H.8327 was an exception and almost the same could be said of J.8877. G.741 laid during her pullet year but not during the second season, at which time the disease began to develop. H.8328 and H.8330 laid only during their second season; presumably the active stage of the disease had passed by then.

Visibly diseased hens, mated to a normal looking cock, can produce affected chicks, as well as others showing nothing unusual—refer to Table 5.

On one occasion we mated a cock and a hen, both of which were diseased. From the union we obtained six fertile eggs, but none hatched. Of course, we can deduce nothing from the fact that all the germs died.

It may be argued that osteopetrosis is due to an infection picked up after birth. It is our practice never to brood chicks together, that have been hatched on different dates, and if we look at the dates of birth of the affected progeny of cock 2899 and hen E.153, for instance, it is apparent that all chicks from all hens must be equally exposed to any infection, unless of course the infective agent is present in the egg. It is possible that the

transmissible agent discovered by Jungherr and Landauer is present in the chick at birth, but the evidence about to be presented shows that a susceptibility to the disease probably has to be inherited before symptoms can develop.

To the characteristics of osteopetrosis observed by others, we would add that the plumage is inclined to look rough, instead of lying smoothly on the body, and the sexual development is noticeably retarded. In common with others, we have found that the affected bones do not fracture easily. Indeed they are very hard, like ivory. This contrasts with the frequency with which fractures characterise the disease in man.

THE FAMILIAL INCIDENCE OF THE DISEASE.

In Tables 2 and 3, which should be studied along with Tables 4 and 5, we show how strikingly this very rare disease can affect individual families. No cognisance is taken in Table 2 of the results of the special matings made in 1941, because the affected progeny on that particular occasion were begotten of hens showing the disease. This special breeding pen will be discussed later. All the parent cocks and hens listed in Table 2 looked perfectly normal.

If susceptibility to osteopetrosis is a manifestation of a simple recessive rather than just a recessive character, and if the transmissible agent of Jungherr and Landauer was always present to take advantage of its opportunities, we would expect the cases enumerated in Table 2 to have numbered 43, i.e. 25 per cent. of 173. It is interesting to note that during the peak year of 1940 there were 23 cases out of 85 chicks retained, which is a remarkably good fit. This evidence is highly suggestive, but we are fortunately able indirectly to corroborate it. Cases could have resulted only from mating carriers, as birds with the disease were never put in our ordinary breeding pens. Carriers can result only from mating cases with normal birds or carriers (and this, of course, was never done) or from mating carriers with normals. Only one fowl out of 698 cocks and hens in the breeding pens had a brother or sister that showed the disease. So when numbers of breeding birds were selected at random from normal-looking fowls which were, with a single insignificant exception, not the brothers and sisters of cases and which presumably arose from the union of carriers and true normals, the breeding pens could be expected to house both carriers and normals representing the tainted families, in equal numbers; and this is what we found, for 16 hens mated to certain cocks gave rise to cases, while 17 sisters of these tainted or carrier cocks and hens, when mated to the same carrier cocks, produced only normal looking progeny. We have not taken into consideration the two sisters, which were mated to their brother, and produced only one chick each.

We realise that definitely to prove susceptibility to osteopetrosis to be a unifactorial recessive, it would be necessary to get only osteopetrotic chicks from the mating of two affected birds, and carriers and visibly diseased chicks in equal numbers from the union of a heterozygous normal with a clinical case. As mentioned elsewhere, we attempted the first type of mating but secured no progeny, for a reason so far undetermined. The second type of mating was also essayed (Table 5), but too few visibly diseased chicks were hatched. The second failure may have been due to insufficient exposure of inherently susceptible birds to the causal agent described by Jungherr and Landauer.

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The above facts, supporting the idea of an inherited susceptibility to the disease determined by the genes, remain to challenge any contention that all our cases of this rare misfortune were due simply to an infection acquired in the egg from the mother, or picked up after hatching, such as in the case of bacillary white diarrhoea.

It is thus only reasonable to suppose that susceptibility is due to a recessive, if not a unifactorial recessive.

DIRECT ATTEMPTS TO PROVE SUSCEPTIBILITY A SIMPLE RECESSIVE CHARACTER.

In order to put our suspicions, that susceptibility might be a simple recessive character, to a final test, we assembled the breeding pen represented in Table 5. Cock 2899 was used because he had already sired 17 cases. Let us analyse the results. There was never any proof at any time that G.625, H.744, G.793 and H.8305 were not completely normal in their genetic make-up, and so their failure to produce cases was not surprising. E.903 was a known carrier, but left us with only two chicks, too small a number to allow inferences to be drawn; the same thoughts were entertained about H.8328 and H.8330.

So we are left with H.8327. This mating of a clinical case with a carrier should have given equal numbers of carriers and clinical cases. In other words, seven of the fifteen chicks might reasonably have had osteopetrosis, and yet we found only 2. But just as it is possible to find thirteen or fourteen boys in a family of fifteen, so it is possible to find less than the expected number of fowls with the genetic constitution of a flagrant case. Another feasible explanation, of course, is that the susceptible birds existed, but that the transmissible agent reached only 2.

This experiment solved nothing and the fact that 8 other hens mated to 2899 yielded no progeny only served to emphasize the difficulties attendant on work of this nature.

The second abortive attempt involving the mating of a diseased cock with an affected hen has already been mentioned.

OSTEOPETROSIS IN MAN.

Jungherr and Landauer are the only investigators who have made a thorough study of the histopathology of *osteopetrosis gallinarum*, and they have claimed that the picture in the fowl is essentially that of the disease in man. So it will be profitable to discuss the condition in the human being, where it also passes under such names as marble bones, *osteosclerosis fragilis generalisata*, congenital osteosclerosis and Albers-Schönberg's disease.

The most conspicuous thing about osteopetrosis in man is its rarity. Not more than 150 cases have so far been recorded. Both sexes are equally prone to it. The disease may begin in early intra-uterine life and be fully developed at the time of birth. Pirie actually diagnosed the condition in a foetus by X-raying the mother. Osteopetrosis may be found at all ages; even a woman of seventy-two revealed the abnormality.

The disease is characterised essentially by increased thickness and density of the cortical and spongy portions of the entire osseous system. The base of the skull, the bodies of the vertebrae and the long bones are these

generally most affected. The dense compact bone encroaches on the medullary cavity, which sooner or later becomes almost entirely obliterated. These areas of sclerosis sometimes appear to be of a uniform density throughout, or they may show transverse lines of rarefaction. One suspects that a case examined very fully by Pirie was so characterised by rarefaction that he found the bones to be of the nature of chalk, and he suggested that the bones should be called chalky bones instead of marble bones. However, all other investigators have testified to the excessive weight, hardness and inelasticity of the bones.

Due to the frequency with which rarefaction occurs, even in very limited areas of the bone, the patients are particularly liable to fracture their limbs and hips as a result of slight injuries. Sometimes a fracture is unaccompanied by pain, and no one is aware of it until the bone is X-rayed. Most cases of osteopetrosis would never be suspected, especially in adults, were the fractured limbs not examined roentgenographically. As it is now customary to X-ray the relatives of cases, more and more instances of the disease are being brought to light.

Roentgenographically, the affected bones appear diffusely opaque and heavy and the finer markings are lacking. The shadow of the marrow cavity tends to disappear.

The disease is never confined to a single bone or to an isolated section of the body. It is widespread in its distribution, but often some bones such as the skull escape.

While the diaphysis of a long bone of the fowl is the region showing the severest changes, the site in man is by no means so fixed. A few illustrations may be given to make this clear. In McPeak's cases there was a definite increase in diameter of the femur, beginning at the juncture of the middle and lower thirds and extending to the supracondylar region, at which point the bone returned to its normal diameter. An increase in diameter was also noted in the lower end of the tibia.

Ghormley examined an eight years old boy and found the cortex of the ribs and the narrow cavity in places apparently obliterated. There was definite thickening of the cortex of all the long bones, more marked in the femora and humeri, and the thickening was greater in the proximal portion of each bone, than in the distal.

Ellis contends that the contour of the bone is not altered by the sclerosis, although clubbing of the posterior clinoid process and of the ends of the long bones, occurs sufficiently often to be regarded as the rule rather than the exception. Ellis thinks the clubbing is not directly due to the sclerosis, for in his two cases the main area of sclerosis occurred in the middle third of the shaft of the long bones and completely outside the area of clubbing. He believes that the site of sclerosis within the bone varies considerably in different cases, and that multiple cases in any one family tend to show a uniform set of lesions. He admits that several authors have insisted that the areas of sclerosis appear at the ends of the long bones. The radiological report on Ellis' two cases stated that the ends of the diaphyses of the long bones were greatly expanded with cortical thickening.

* Many cases show a slight anaemia, usually of the hypochromic type. There is no conclusive evidence that the chemical composition of affected bones deviates from the normal.

The picture presented so far applies mainly to what may be called a benign type of the disease, which is seen in most adults and many children. The people are to all intents and purposes normal, except for a tendency to break bones easily. In infants a far more dangerous and lethal form of the disease is often encountered. All the lesions occur that have so far been enumerated. In addition, there may be some retardation in the longitudinal growth of bone, and the general build may be stocky. The child may have a pigeon breast and a square forehead. Dentition is often arrested and the teeth tend to decay. Owing to the contour of the face, there is nearly always a purulent rhinitis. Sexual development is delayed. Severe damage to the base of the skull often leads to partial occlusion of the foramina through which the optic and other nerves emerge. Thus we find blindness due to optic atrophy, and nystagmus and deafness and facial palsies and sometimes hydrocephalus and not infrequently defective mentality. The prognosis in these severe cases is virtually hopeless.

Some investigators have found leukaemia, carcinosis and sarcomatosis associated with human osteopetrosis, but there is no suggestion that the disease is necessarily connected with these malignant conditions.

The cause of the disease in man is unknown, but the familial incidence of the condition has often been noted and a few examples will be given. Ghormley found lesions in a father and his son, the boy's mother was normal. Pirie's cases included a mother and her son and two daughters, and it was this boy who was examined roentgenographically while still in utero. McPeak found three generations involved— a grandmother, her two daughters and five children (2 boys and 3 girls) of one of these daughters. A sister of the five affected children was normal. A daughter and a son of the other affected daughter of the grandmother were also normal.

Clifton, Frank and Freeman's child was born of relatives who had married. They state that Harnapp found the disease in a father and five of his seven children.

Ellis' two cases were brothers and their parents were second cousins.

Higinbotham and Alexander found an adult family of two brothers and two sisters all affected, and they also showed prognathism and syndactylism.

Other workers' observations have been reviewed by McCune and Bradley. In Kudijawtzewa's series of cases, both parents had been married before, the man having three normal children and the woman one. By their consanguineous marriage, these people had five children, two of whom died in infancy and three of whom had the disease. D'Istria had a similar experience. The man had three normal children by the first marriage. Then he married a cousin and three children died in infancy and one more was mentally defective and a fifth had osteopetrosis. Lorey and Reve diagnosed the disease in three sisters, and a cousin of these girls was found to be affected by Sick. Lauterburg saw the condition in two brothers. Cohn and Salinger found three children diseased in one family; Frank had the same experience with another.

All these observations are very impressive but may, nevertheless, have their value enhanced if we allude to the studies of Herzenberg and Lewit, who uncovered two pedigrees to indicate that marble bones are recessive in man. Some workers have suggested that the susceptibility to this very rare disease of human beings is a simple recessive character. This may or may

not be so. If it is so, we must assume that the grandmother and one of her daughters mentioned by McPeak, must both have married men, who were at least carriers. Such a coincidence is not impossible, even if it is improbable. Only extensive studies in the future can settle the point.

DISCUSSION.

In this article we have discussed the spontaneous occurrence of *osteopetrosis gallinarum* in the Onderstepoort flock of White Leghorn fowls. Our motive in maintaining this flock is to attempt to breed a strain of fowls highly resistant to neoplastic conditions which, as we know, are responsible for such appalling economic losses in the poultry industry. We have described the criteria for the selection of breeding birds, and we have indicated the considerable degree of success already achieved.

Except for assembling the one breeding pen in 1941, we made no effort to increase the incidence of osteopetrosis. Rather did we prefer to see just what the ravages of the natural disease would be. We also made no attempt to search for the transmissible agent found by Jungherr and Landauer. It remains for future workers to correlate their findings with ours.

We think that we have unearthed very telling evidence in support of our belief that the susceptibility to *osteopetrosis gallinarum* is a recessive character, maybe a simple recessive one. No cases appeared in the birds hatched in 1942, but of those brought out in 1943, two, a brother and a sister, have already developed lesions, and this is additional support for our contention.

It is by no means improbable that the appearance of symptoms depends on an inherited susceptibility, together with the presence of a transmissible agent. Whether this agent is of exogenous or endogenous origin, matters little at this stage. Obviously the next step in the investigation of the disease is to see whether the dual cause hypothesis is correct. That Jungherr and Landauer could not produce lesions in more than 20 per cent. of their experimental chicks rather indicates that susceptibility is very much conditioned by one or more factors, possibly of a genetic nature.

Even if we concede that the histopathological picture is the same as that in osteopetrosis of man, we cannot be sure that the two diseases are identical in all essential respects. Both conditions are very rare. Both tend to affect a number of subjects in each family concerned. In the two diseases both males and females are susceptible and an affected parent may give rise to diseased progeny. Both are characterised by retarded sexual development. The affected bones are dense, heavy and hard, and lack elasticity and the marrow cavity is inclined to disappear. Diseased subjects of both species sometimes show anaemia.

A few differences are also well worth noting. The skull, vertebrae, and phalanges of the fowl do not seem to suffer, as they almost invariably do in man. No one has observed the increase in temperature over an affected human bone, such as Jungherr, Landauer and we have repeatedly witnessed in the case of the fowl. It is the diaphysis of the long bone of the hen that is so badly involved. From the descriptions of bones at our disposal, it is obvious that such a definite site for the development of the abnormality does not exist in the human being.

In man the contour of the diseased bone remains more or less unaltered, whereas in birds there are hard, rough, osseous excrescences above the

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surface and very often the overall diameter of the bone is two to four times the normal. Fowls show no clubbing at the ends of the long bones.

Nobody has seen a fractured bone in an affected fowl, but this may be due to the fact that birds do not fall as heavily as people.

On the whole, the points in common seem to outweigh those that are dissimilar and, for the time being at least, we should join with Jungherr and Landauer in thinking that osteopetrosis in the fowl is probably the same as marble bones in man.

SUMMARY.

Thirty-nine spontaneous cases of osteopetrosis have been studied in the Onderstepoort experimental flock of fowls, and the disease has shown a striking tendency to affect some families more than others. Evidence has been advanced indicating that a susceptibility to the disease depends on a recessive character. There is also some indirect evidence that susceptibility depends on a unifactorial recessive.

Malignant conditions, such as leucosis and carcinosis, were not found more frequently in fowls with osteopetrosis than in the flock as a whole.

Families stigmatized with osteopetrosis almost never provide birds worthy of inclusion in a high class breeding pen. We have stated the grounds on which a hen should be admitted to a good breeding pen, and we have indicated what success has crowned our efforts to evolve a strain of fowls resistant to neoplastic conditions, while being also highly desirable in all other respects.

The literature dealing with osteopetrosis in fowls and man has been reviewed and the points of similarity and dissimilarity have been discussed.

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Fig. 1.- White Leghorn cockerel with osteopetrosis



Fig. 2.



Fig. 3

Fig. 2 Leg of fowl showing how the bones are thickened

Fig. 3.--White Leghorn pullet with osteopetrosis

TABLE 1.

Summary of Cases of Osteopetrosis, Neurolymphomatosis, Leucosis, Carcinosis and other Malignant Disease Diagnosed in the Onderstepoort Flock of Pedigreed White Leghorns from 1937 to 1942.

Year Hatched.	Autopsies.	Cooks in Breeding Pens.	Hens in Breeding Pens.	Osteopetrosis.	Osteopetrosis plus Neurolymphomatosis.	Osteopetrosis plus other malignant conditions.	Neurolymphomatosis plus Leucemia.	Other malignant conditions.	Total Neurolymphomatosis plus other malignant conditions.
1937. D fowls.....	431	8	95	1	—	1	36 8.4%	19 4.4%	185 42.9%
1938. E fowls.....	755	10	109	1	—	—	42 5.6%	16 2.1%	311 41.2%
1939. G fowls.....	931	10	135	2	—	3	57 6.1%	22 2.4%	371 39.8%
1940. H fowls.....	1,119	15	160	14	2	7	21 1.9%	6 0.5%	239 21.4%
1941. J fowls.....	777	15	144	6	—	—	3 0.4%	1 0.1%	84 10.8%
1942. K fowls.....	418	9	76	—	—	—	6 1.4%	—	32 7.7%

Actually only 55 cocks were used from 1937 to 1941. Three cocks served for two years. During the same period 643 hens were mated.

There are still 81 J and 171 K fowls alive and, as they are all worthy of inclusion in the breeding pens, the figures of 10.8 per cent. and 7.7 per cent. given for "Total neurolymphomatosis plus other malignant conditions" will ultimately be improved upon.

On birds hatched from 1937 to 1941, 4,013 autopsies have been performed and 29.7 per cent. of these showed neurolymphomatosis and/or some other malignant condition.

Of 37 cases of osteopetrosis, 13 or 35.1 per cent. showed associated neurolymphomatosis, carcinosis, etc.

Thus, there is no significant increase of leucosis, etc. in fowls suffering from osteopetrosis.

FAMILIAL INCIDENCE OF "OSTEOPETROSIS GALLINARUM

TABLE 2.
Matings that Produced Osteopetrosis gallinarum.

Year.	Cock.	Hen.	Cases.	Chicks retained over age of two months.
1937.....	B. 536.....	A. 237.....	1	16
		B. 416.....	1	12
1938.....	520.....	B. 462.....	1	10
1939.....	42.....	E. 96.....	1	3
1939.....	317.....	E. 119.....	1	16
		E. 400.....	1	14
		E. 919.....	2	4
1940.....	2572.....	E. 223.....	3	12
1940.....	2788.....	E. 903.....	1	12
		E. 985.....	2	14
1940.....	2899.....	E. 69.....	4	9
		E. 153.....	7	18
		E. 268.....	4	14
		E. 406.....	1	4
		E. 440.....	1	2
1941.....	307.....	G. 959.....	3	13
	8.....	16.....	34	173

Cock 2788 and G. 959 were brother and sister.

Hens E. 69, E. 268 and E. 440 were full sisters.

Nineteen full sisters of hens and cocks producing the disease were mated to the above eight males without revealing cases in their progeny, but only one chick was retained from each of two of these nineteen, and so we have reliable evidence concerning the progeny of only seventeen.

TABLE 3.

Familial Incidence of Osteopetrosis in 1940, the Year of its Greatest Frequency.

Sire.	Dam.	Type of Offspring.	Progeny Retained over Age of 2 Months.		
			No.	Osteopetrosis.	
				No.	Per cent.
2560 Resistant Progeny.....	10 Hens	Resistant	100	0	0
2581 Resistant Progeny.....	10 Hens	Resistant	130	0	0
2596 Resistant Progeny.....	7 Hens	Resistant	54	0	0
2626 Resistant Progeny.....	14 Hens	Resistant	98	0	0
2671 Resistant Progeny.....	10 Hens	Resistant	95	0	0
2683 Resistant Progeny.....	12 Hens	Resistant	138	0	0
2685 Resistant Progeny.....	10 Hens	Resistant	92	0	0
2734 Resistant Progeny.....	8 Hens	Resistant	60	0	0
2812 Resistant Progeny.....	7 Hens	Resistant	13	0	0
2829 Resistant Progeny.....	7 Hens	Resistant	85	0	0
3102 Resistant Progeny.....	9 Hens	Resistant	64	0	0
3139 Resistant Progeny.....	9 Hens	Resistant	69	0	0
J 5637 Resistant Progeny.....	15 Hens	Resistant	156	0	0
2572 Susceptible Progeny.....	8 Hens	Resistant	55	0	0
	E 223	Susceptible	12	3	25
2788 Susceptible Progeny.....	10 Hens	Resistant	61	0	0
	E 903	Susceptible	12	1	8.3
	E 985	Susceptible	14	2	14.3
2899 Susceptible Progeny.....	6 Hens	Resistant	72	0	0
	E 69	Susceptible	9	4	44.4
	E 153	Susceptible	18	7	38.9
	E 268	Susceptible	14	4	28.6
	E 406	Susceptible	4	1	25
	E 440	Susceptible	2	1	50

Total chicks from susceptible hens = 85.

Total cases from susceptible hens = 23.

The above table emphasizes how susceptible families are to osteopetrosis gallinarum, while the great majority of families seem to be resistant.

FAMILIAL INCIDENCE OF "OSTEOPETROSIS GALLINARUM".

TABLE 4.
Detailed Summary of the Understepport Cases of Osteopetrosis gallinarum.

Fowl.	Sex.	Date Hatched.	Sire.	Dam.	Eggs Laid.	Age at Disposal in Days.	Remarks.
D 734	Female	7/9/37	B 536	A 237	36	327	Osteopetrosis. Lymphoid leucosis of liver, spleen and lungs.
671	Male	25/8/37	B 536	B 416	—	?	Osteopetrosis.
349	Male	25/8/38	520	B 462	—	?	Osteopetrosis.
3955	Male	22/9/39	42	E 96	—	230	Osteopetrosis. Killed.
G 746	Female	18/8/39	317	E 119	32	298	Mild osteopetrosis. Erythroleucosis.
G 741	Female	1/9/39	317	E 400	76	899	Osteopetrosis. Cystic vestigial remains of the right Mullerian duct. Laid only during pullet year. Disease first diagnosed at age of 818 days. This was the oldest case diagnosed.
2786	Female	1/9/39	317	E 919	0	140	Osteopetrosis. Lymphocytoma of the gizzard.
G 141	Female	1/9/39	317	E 919	9	302	Osteopetrosis. Lymphoid leucosis of liver. Embryonal nephroma of kidney.
92	Female	30/8/40	2572	E 223	0	369	Osteopetrosis. Lymphocytoma in abdomen on lateral surface of cloaca. Lymphocytematosis of myocardium.
H 651	Female	13/9/40	2572	E 223	6	495	Slight osteopetrosis. Killed.
H 8327	Female	30/8/40	2572	E 223	217	575	Moderate osteopetrosis. Lymphoid leucosis of liver and spleen. Haemorrhage into an ovum. Aerocystitis of one abdominal air sac. Mother of the cases Male 3771 and Male 4118.
1668	Female	20/9/40	2788	E 903	0	263	Mild osteopetrosis. Lymphoid leucosis of liver and kidneys.
53	Male	23/8/40	2788	E 985	—	141	Osteopetrosis. Bullied to death by other fowls.
1255	Female	13/9/40	2788	E 985	0	218	Osteopetrosis.
1463	Female	20/9/40	2899	E 69	0	176	Osteopetrosis. Sexually retarded. Killed.
1464	Female	20/9/40	2899	E 69	0	187	Osteopetrosis. Carcinoma of ovary. Killed.
4842	Female	23/8/40	2899	E 69	0	204	Osteopetrosis. Sexually retarded. Killed.
4844	Male	23/8/40	2899	E 69	—	48	Osteopetrosis. Killed. This was the youngest case.
625	Female	6/9/40	2899	E 153	0	201	Osteopetrosis. Killed.

TABLE 4.—(continued).

Fowl.	Sex.	Date Hatched.	Sire.	Dam.	Eggs Laid.	Age at Disposal in Days.	Remarks.
1055	Female	13/9/40	2899	E 153	0	181	Osteopetrosis. Chronic cardiac dilatation. Cirrhosis and venous stasis of liver. Deposit of fibrin on surface of liver.
H 8310	Female	20/9/40	2899	E 153	0	434	Osteopetrosis.
H 8328	Female	20/9/40	2899	E 153	18	484	Osteopetrosis. Moderate anaemia. No eggs were laid in the pullet year.
H 8329	Female	16/8/40	2899	E 153	0	359	Marked osteopetrosis. Chronic asplinitis. Lymphocytomata of ovary and peritoneum and of skin at the side of the tail.
H 8330	Female	30/8/40	2899	E 153	61	496	Osteopetrosis. Myeloid leucosis. No eggs laid in pullet year. Mother of Male 3354.
4846	Female	23/8/40	2899	E 153	0	204	Osteopetrosis. Sexually retarded. Killed.
1476	Female	20/9/40	2899	E 268	0	187	Osteopetrosis. Neurolymphomatosis of the right brachial and right sciatic nerves.
H 8294	Female	6/9/40	2899	E 268	0	489	Osteopetrosis and neurolymphomatosis.
H 8297	Female	30/8/40	2899	E 268	0	281	Mild osteopetrosis. Multiple haemangiogenous endotheliomata of the skin, left kidney, lungs and liver. Chondromata of the skin and subcutis.
H 8336	Female	20/9/40	2899	E 268	0	310	Mild osteopetrosis.
646	Female	6/9/40	2899	E 406	0	225	Osteopetrosis. Killed.
1500	Female	20/9/40	2899	E 440	0	225	Osteopetrosis. Sexually retarded. Hepatic cirrhosis with cardiac dilatation, hydropericardium and pulmonary oedema and intestinal catarrh.
3354	Male	18/8/41	2899	H 8330	—	162	Very severe osteopetrosis. Mother had the disease.
3771	Male	25/8/41	2899	H 8327	—	317	Marked osteopetrosis. Killed. Mother had the disease.
4118	Male	1/9/41	2899	H 8327	—	199	Slight osteopetrosis. Disease more developed in left leg. Septic iridocyclitis of right eye. Killed. Mother had the condition.
4406	Female	8/9/41	307	G 959	0	181	Osteopetrosis. Killed.
J 8877	Female	15/9/41	307	G 959	140	449	Slight osteopetrosis.
J 9080	Female	15/9/41	307	G 959	64	449	Slight osteopetrosis. Killed.

FAMILIAL INCIDENCE OF " OSTEOPETROSIS GALLINARUM

TABLE 5.

Special 1941 Mating—Cock 2899.

Hen.	Remarks.	Chicks Retained.	Pullets Leg-banded.	Cases.
G 625	Sister of cock 2899.....	12	5	0
H 744	Sister of cases 625, 1055, H 8310, H 8328, H. 8329 H 8330 and 4846	7	3	0
G 793	Sister of cock 2899.....	5	4	0
E 903	Had produced a case when mated to cock 2788....	2	0	0
H 8305	Sister of cases 625, 1055, H 8310, H 8328, H 8329, H 8330 and 4846	14	9	0
H 8327	Had osteopetrosis.....	15	9	2
H 8328	Had osteopetrosis. Daughter of cock 2899.....	1	0	0
H 8330	Had osteopetrosis. Daughter of cock 2899.....	1	0	1

Eight other hens were also mated, but either laid no eggs or gave no progeny.

A Study of the Compressibility of Wool, with Special Reference to South African Merino Wool.*

By C. M. VAN WYK, Wool Research Section, Onderstepoort.

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Promoter: Prof. S. M. Naudé).

THE COMPRESSIBILITY OF WOOL.

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“ It is probable ”, state Frölich, Spöttel and Tänzer (1929) “ that ever since wool has been converted into fabric, it has been recognised that the mechanical properties of wool, besides its fineness, play an important if not decisive role in technical processing ”.

It follows that the physical properties of the raw material are of fundamental importance to both producer and manufacturer, although the points of view of the two differ in many respects.

In production practice the estimation of fleece characteristics depends on the senses of sight and touch, and consequently suffers from the errors inherent in human estimation. With the advent of research, exact methods of measurement have been developed, and the results being obtained are laying stress on the necessity of employing such exact methods in breeding practice.

Of the properties of wool, the dimensional attributes of the fibre, and its behaviour under longitudinal stress have received most attention. On the one hand, the factors which influence the production of these properties are being investigated, and on the other hand, the knowledge of the structure of the wool fibre has advanced to a remarkable extent. Other properties, though admitted to be of great importance, have received less attention. One of these is the elastic behaviour of wool in bulk, and its neglect must be considered somewhat surprising in view of the extent to which both producer and manufacturer rely on tactual examination in wool judgment, and the stress

generally laid in production on various characteristics known as "quality", "substance", "handle", "harshness", and others which may be expected to involve the compressibility of wool.

The present study is concerned with the resistance offered by the fibre mass to compression, and it is considered mainly from the point of view of the producer. Since the first essential to the study of any property is its expression in arithmetical terms, the first part of the investigation involves a study of the method of measurement, next the elastic behaviour of wool under compression, and finally the arithmetical expression of resistance to compression.

In the second part a study has been undertaken of the relation between the compressional characteristics and the more obvious fleece and fibre attributes, and of the factors which influence the compressibility. In view of the lack of knowledge of the subject, it has been the aim to investigate several such factors and attributes, rather than to study a few of these exhaustively, for by this method a better foundation for future experimentation could be laid. In the discussion of the results, an attempt has been made to show how the compressibility can influence breeding practice.

PART I.

THE MEASUREMENT OF COMPRESSIBILITY, AND THE ELASTIC BEHAVIOUR OF WOOL IN BULK.

A. THE MEASUREMENT OF COMPRESSIBILITY.

(a) *Historical.*

In practice both sheep breeder and wool manufacturer resort to tactual examination for estimating those characteristics of a wool sample which depend upon its compressibility and resilience. The resistance offered by the sample to compression by hand, and its mode of recovery to its original form when released, are taken as the bases of judgment. On the live animal a staple is often separated from the rest of the fleece, and its ability to remain erect is observed.

A method in use for comparing the pliability of single fibres, according to Heyne (1924), consists in blowing gently towards them and noting the extent to which individual fibres respond. Frölich, Spöttel and Tänzer (1929) state that in sheep breeding practice the pliability of a fibre is estimated by holding a 2 cm. length erect between the fingers and blowing towards it until it lies horizontally. From the force necessary, and the extent to which the fibre rises after cessation of the blowing, the pliability of the fibre is estimated.

Such methods are, however, subjective, and the results are dependent on individual opinion and cannot be expressed in arithmetical terms. From time to time, therefore, methods have been devised for studying the elastic behaviour of wool under compression more precisely.

Herzog (1916) studied the properties of materials utilised for upholstery, particularly horsehair. He regarded compressibility and resilience as of supreme importance, and devised an apparatus for their measurement. The

sample was compressed by a falling weight, and the compressibility and resilience were estimated from the successive minimum and maximum heights of the material. Sommer (1936) employed the same method, using different coefficients for expressing the relevant characteristics. His determinations were extended to include mixtures of fibre types, and the effect of impregnation with rubber.

A method of comparing the pliability (*Schmiegsamkeit*) of tops was devised by Kraus (1922). A certain length and weight of top was subjected to a torsional couple while under a longitudinal tension of 75 gm. The number of turns in the top and the resulting reduction in length were noted.

M. and J. Eggert (1925) enclosed the sample to be tested in a small rubber balloon. The balloon was in turn enclosed in a glass vessel with the neck protruding. After the spaces inside and outside the balloon had been partially exhausted to the same pressure, compression was effected by allowing small quantities of air into the outer glass vessel. Manometers indicated the pressures inside and outside the balloon, and the difference between them was taken to represent the pressure acting on the wool. The volume of the balloon was determined at one pressure, and since the quantity of air in the system containing the wool remained constant, the volume at other pressures was calculated by Boyle's Law. An equation containing two constants was assumed to represent the relation between pressure and volume. One of the constants was regarded as a measure of the pliability of the wool, and the other as a measure of its softness.

The possibilities of the balloon method as a method of measuring resilience were later investigated by Winson (1932). He took as a measure of the resilience of the wool the area of the hysteresis loop formed between the compression and the release curves, since his results indicated that this quantity corresponded to the trade impression of "springiness".

The balloon method, in the form employed by the Eggerts and by Winson, suffered from the serious defect that the relative humidity of the air surrounding the wool was uncontrolled and variable. This defect was overcome by Pidgeon and van Winsen (1934). The balloon was compressed by liquid pressure, the neck of the balloon was left open and the enclosed sample could be maintained in equilibrium with any desired relative humidity. While the primary object of the investigation was to study the effect of sorbed water on the compressibility of asbestos, a wool sample and a cotton sample were included for comparison.

The relation between the harshness of two yarns and their compressibility was investigated by Larose (1934). Both the balloon method, as employed by Winson, and a method devised by him were used. In his method the sample was placed in a steel cylinder and compressed by a piston attached to a calibrated spring. Both methods led to the conclusion that the harsher of the two yarns offered the greater resistance to compression, and the effect of dyeing was in each case to increase both the harshness and the resistance to compression.

An instrument, known as the "Pendultex", designed by Henning (1934, 1935), was based on a different principle. A swinging pendulum was made to compress the sample under test, and the compressional resistance of the sample was measured by the consequent damping of the pendulum. The number of swings during which the amplitude decreased from one value to another was recorded on a counter, and the greater the resistance of the

sample to compression, the smaller was the number of swings recorded. The instrument was stated to be suitable for the study of compressibility at any stage from the raw material to the finished product, and was shown to be valuable for following the changes produced by the various processes.

In connection with his researches on felting, Schofield (1938) made a compression test on wool in the form of a sliver, by filling a wooden box with the sliver and placing weights on a lid which fitted into the box exactly.

The methods and instruments described above were all employed or were suitable for determinations on the raw material. To them may be added those of a number of investigators who studied the elastic properties of the finished product, particularly the resistance to bending and the resistance to compression. The work of these authors will not, however, be here considered, since the present investigation is confined to a study of the raw product.

A compressional method of estimating the clean yield of fleeces was developed by Burns and Johnston (1936, 1937) and Johnston and Gray (1939). A high correlation between the percentage of clean wool and the volume occupied by a greasy sample under a high pressure was obtained.

The present study is based on results obtained with the "Pendultex" instrument, but certain aspects of the elastic behaviour of wool in bulk have been investigated by a static cylinder and piston method.

(b) *The "Pendultex" method of determining the compressibility of textiles.*

(i) *Description of the instrument.*

Figure 1 illustrates the essential features of the "Pendultex" instrument designed by Henning (1934) for determining the compressibility of wool and other textiles.

A heavy pendulum, A, is suspended at B and moves over a scale C, graduated in degrees. The pendulum is extended upwards and carries two levers, D and E, which are held in position by the springs F. A light bar G is suspended at its midpoint on the same axis B, but is free to move independently of the pendulum. To one end of the bar G is attached an arm carrying a piston, H, which fits into the stationary compression compartment J. The other end of the bar carries two balancing nuts M.

The wool to be tested is placed in the compression compartment J, and the pendulum is raised to a pre-determined angle and then released. At the lowest position of the pendulum, the lever E engages the piston, which is forced into the compartment J where it compresses the sample. When the lever D reaches the stop K it is forced outwards and draws the lever E off the piston. The piston thus released jumps back to its original position under pressure by the sample. On the return half of the swing the pendulum moves freely.

The process is repeated during each successive swing. The extent to which the sample is compressed is determined solely by the position of the stop K, and is independent of the amplitude, above a certain value of the latter. The position of the stop K can be varied in order to produce different degrees of compression.

The resistance offered by the sample has a damping effect on the pendulum, and is measured by the number of swings during which the

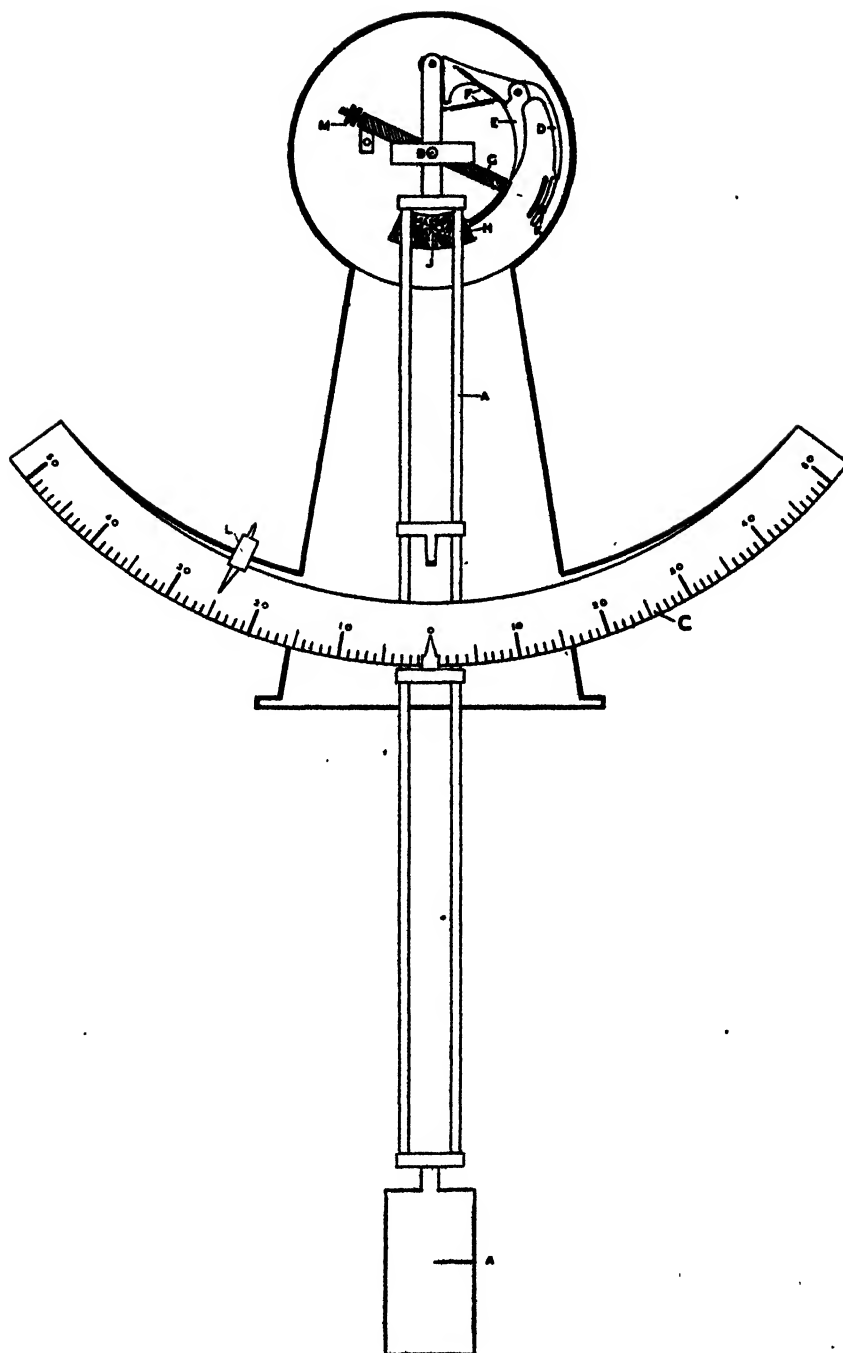


FIGURE 1.—The "Pendultex" instrument designed by Henning, for determining the compressibility of wool and other textiles.

amplitude decreases from its initial value to a final fixed value. At the latter angle, a counter L is attached to the scale C and records the number of swings made by the pendulum.

It may be considered that a method of recording the reduction in amplitude during a swing directly is to be preferred to the less direct method of counting the number of swings between two fixed values of the amplitude. It should be borne in mind, however, that the apparatus was designed for routine determinations, and the experience gained during the course of the present study confirmed the designer's view that the method of counting the number of swings is far simpler, requires less manipulation, and gives a more reliable result.

The designer as well as the manufacturer of the instrument state that for comparative purposes the number of swings recorded by the counter is a sufficient indication of the resistance offered by the sample. Since results obtained in this form are dependent upon the constants of the pendulum and the arbitrarily chosen initial and final amplitudes, they cannot be compared with results obtained by other methods. The most serious objection to this method of presenting the results is, however, the non-linear relationship between the number of swings and the work done in compressing the sample, making it a matter of extreme difficulty to assess the true magnitude of the differences between samples.

The first object in the investigation was to find a relation between the work done in compressing a sample and the total number of swings of the pendulum between the two fixed amplitude values. The following is a summary of the procedure adopted:—

The work done in compressing the sample was estimated from the loss of potential energy of the pendulum during one swing. If M is the mass of the pendulum in Kg., and h the distance from the centre of gravity to the point of suspension, the potential energy at amplitude θ is given by $Mh(1 - \cos \theta) = 2Mh(\sin^2 \frac{\theta}{2})$ in Kg.cm.

During the first swing the amplitude is reduced from θ_0 to θ_1 , hence the loss of potential energy during the first swing is $2Mh(\sin^2 \frac{\theta_0}{2} - \sin^2 \frac{\theta_1}{2}) = 2Mh(A_0^2 - A_1^2)$, where $A = \sin \frac{\theta}{2}$. Since A_0 is fixed, the problem is that of finding A_1 .

Instead of determining A_1 directly, the amplitudes after successive swings were noted, and the equation

$$\sin \frac{\theta}{2} = A_0 - Bn + Cn^2 \dots \dots \dots (1)$$

where n was the number of swings, was fitted to the observations, whence A_1 could be calculated.

If N was the total number of swings, then

$$A_1 = A_0 - B + C,$$

$$A_N = A_0 - BN + CN^2.$$

Eliminating B ,

$$A_1 = \frac{A_N + (A_0 - CN)(N - 1)}{N} \dots \dots \dots (3)$$

Now A_0 and A_N were fixed, and C was found experimentally to be independent of the compressibility of the sample and of the degree of compression. By making 62 determinations, and fitting equation (1) to each, a mean value of C was derived and substituted in equation (3). The only unknowns in equation (3) were then A_1 and N , so that A_1 could be calculated for any value of N . Thus the loss of potential energy of the pendulum during the first swing, given by $2Mh(A_0^2 - A_1^2)$, was known from N , the total number of swings between the two fixed amplitudes.

In order to find the work done in compressing the wool, a correction had to be applied for the natural damping of the pendulum. The correction was evaluated in two stages, (1) the loss of potential energy with the pendulum swinging freely, obtained by observing successive amplitudes as before, and (2) increased loss with load on the piston, obtained by determining the loss of potential energy of the pendulum while extending springs whose load-extension relations were known.

On applying the corrections, the work done in compressing the wool sample could be calculated from the number of swings made by the pendulum between the two fixed amplitude values.

The procedure outlined above is elaborated in the following pages.

The relevant constants of the instrument used in the present study were found by measurement to be as follows:—

v_1 = Total volume of compression compartment = 103.3 c.c.

M = Mass of pendulum = 11.666 Kg.

h = Distance from centre of gravity to point of suspension = 96.25 cm.

Hence the potential energy of the pendulum at amplitude θ was given by $Mh(1 - \cos \theta) = 1123(1 - \cos \theta) = 2246 \sin^2 \frac{\theta}{2}$ Kg. cm.

The amount of wool to be tested was limited to 5 gm., as it was found that the 7 gm. recommended imposed a serious strain on certain parts of the apparatus. The instrument was set up in a room maintained at constant relative humidity and temperature, and all determinations were carried out on samples conditioned in this room.

(ii) *The effect of repeated compression.*

Previous investigators have found that when a wool sample is taken through successive cycles of compression and release, the position of the pressure-volume curve is altered after each cycle, but becomes constant after several cycles. Winson, therefore, made his determinations on the eighth cycle, Pidgeon and van Winsen proceeded to the fourth cycle, while Larose found that constancy was attained after the fifth or sixth cycle.

With the "Pendultex" instrument it was found that the number of swings registered by the counter increased with successive determinations until a constant value was obtained after about the fifth determination, representing at least 150 compressions. The resistance to compression offered by the wool was, therefore, reduced by a diminishing amount. For this reason Henning recommended that successive determinations be made without removal of the wool, until a constant value of the number of swings was obtained.

It was also found that if the wool was removed after each determination and teased out into as loose a mass as possible, exactly the same value could be obtained for a subsequent determination. It was decided, therefore, to base the study throughout on the resistance offered by the wool during the first compression. This quantity was evaluated from the total number of swings, and therefore lost nothing in accuracy compared to the method advocated by Henning. The question will be raised again in the subsequent discussion.

All results which follow must therefore be regarded as having been obtained from the first compression of teased samples.

(iii) *The motion of the pendulum.*

The first step in evaluating the work done in compressing the wool was to express the loss in potential energy during the first swing as a function of the total number of swings between the two fixed values of the amplitude. Two ways were open for accomplishing this object. Either an automatic recorder, such as a strip of waxed paper on the scale and a recording needle on the pendulum, could have been employed for recording the reduction in amplitude during the first swing, or the amplitude after each swing could have been noted, and the loss of amplitude during the first swing calculated by fitting an equation to the observations.

In the present investigation the latter method was employed. It was found to be a matter of extreme difficulty to start the pendulum in exactly the correct plane in every case, so that the first swing gave rather erratic results, while the value calculated from the observed amplitudes after successive swings gave reproducible results.

A study was therefore made of the reduction in amplitude of the pendulum during successive swings, and Figure 2 illustrates three typical cases during the compression of five gm. of three different wools by approximately 50 per cent.

The amplitudes at the end of equal numbers of successive swings were noted, but the difficulty of observing the angles with sufficient accuracy, and the somewhat inconsistent oscillation of the pendulum made it necessary that some method had to be applied for smoothing the data. The most suitable method was the fitting of an equation, and since the theoretical derivation of the equation of motion was impracticable owing to the large amplitudes and the discontinuous nature of the damping forces, an empirical relation had to be found.

In an attempt to express the number of swings as a function of the amplitude, the simple trigonometric and hyperbolic functions, their logarithms, and a large number of combinations of these were tried. The simplest functions failed to give a satisfactory fit, while the more complicated combinations introduced so many constants that a good fit was obtained, but not the desired smoothing.

The introduction of the second power of the number of swings led to the equation

$$\sin \frac{\theta}{2} = A_0 - Bn + Cn^2 \dots \dots \dots (1)$$

where θ was the amplitude, n the number of swings and A_0 the initial value of $\sin \frac{\theta}{2}$. The equation was found to fit the observations closely in the range 48° to 25° , and is represented in each case by the full line in Figure 2. The

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constant C varied little from one sample to another, and bore no relationship to either the compressibility of the samples or the degree of compression. The assumption seemed justified that a simple equation applicable to all cases had been found.

The nature of the equation shows that its range is limited, since in certain cases it predicts no zero value for θ . Replacing $\sin \frac{\theta}{2}$ by its logarithm in equation (1) gave as good results, which in fact differed but little from those of equation (1), but the calculations were more complicated. Equation (1) was used throughout the present study, and it was regarded as conforming to the conditions that it should fit the observations closely, be applicable to all cases, and contain a minimum number of constants.

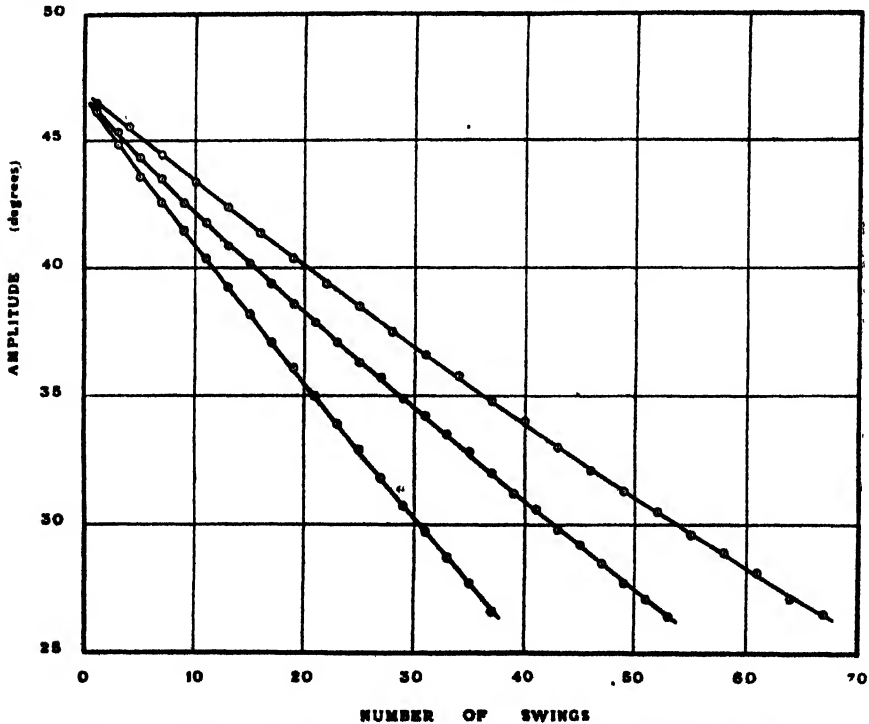


FIGURE 2.—The reduction in amplitude of the pendulum, while compressing 5 gm. of three different wools by 50 per cent.

An added advantage was the ease with which the relevant calculations could be made. For example, the potential energy at an amplitude θ is proportional to $\sin^2 \frac{\theta}{2}$, and the reduction in $\sin^2 \frac{\theta}{2}$ during the swing from θ_n to θ_{n+1} is given by:—

$$\sin^2 \frac{\theta_n}{2} - \sin^2 \frac{\theta_{n+1}}{2} = \sqrt{B^2 - 4A_0C + 4C \sin^2 \frac{\theta_n}{2}} - C \dots \dots (2)$$

Equation (1) was fitted to the observations by the method of least squares, and a method based on that of Gauss was used for solving the normal equations rapidly.

- (iv) *The relation between the loss of potential energy during the first swing, and the total number of swings between two fixed amplitudes.*

The next step consisted in expressing the loss in potential energy during the first swing as a function of the total number of swings between the two fixed amplitudes. For this purpose a series of wool samples was selected, and five gm. of each compressed to various degrees in the instrument, by varying the stop K (Figure 1). In each case the amplitudes at the end of every second, third or fourth swing (depending on the total number of swings) were noted, and equation (1) fitted. By evaluating the constants B and C for each degree of compression of each sample, the loss in potential energy of the pendulum during the first swing was calculated in each case. In all, sixty-two independent determinations were made, no sample being compressed to the same volume more often than once. The circles in Figure 3 represent the values so obtained.

The average relation was found by substituting 1 and N for n in equation (1), and eliminating B from the two equations thus obtained, giving

$$A_1 = \frac{A_N + (A_0 - CN)(N - 1)}{N} \dots\dots\dots(3)$$

Now A_0 and A_N were kept constant, but small variations were found in the values calculated from the sixty-two determinations, variations in A_0 being mainly due to errors in starting the pendulum, and those in A_N being due to the fact that the counter recorded only complete swings. The means of the calculated values of A_0 , A_N and C were

$$A_0 = 0.39769$$

$$A_N = 0.22666$$

$$C = 4.81515 \times 10^{-6}.$$

The employment of a mean value for C was justified by the experimentally found independence of C on either the compressibility or the degree of compression. Substituting in (3), the only unknowns were A_1 and N , so that A_1 could be calculated for any value of N .

The loss in potential energy during the first swing was then given by $2Mh(A_0^2 - A_1^2) = 2246(A_0^2 - A_1^2)$ in Kg. cm. Values so calculated for various values of N are given in Table 1.

TABLE 1.

The loss of potential energy of the pendulum (in Kg. cm.) during the first swing, as a function of the total number of swings, N .

N.	0	1	2	3	4	5	6	7	8	9
30	10.31	9.99	9.70	9.43	9.17	8.93	8.70	8.48	8.28	8.09
40	7.90	7.73	7.57	7.41	7.26	7.12	6.98	6.85	6.73	6.61
50	6.50	6.39	6.28	6.18	6.09	6.00	5.91	5.82	5.74	5.66
60	5.59	5.52	5.44	5.38	5.31	5.25	5.19	5.13	5.07	5.02
70	4.96	4.91	4.86	4.82	4.77	4.72	4.68	4.64	4.60	4.56
80	4.52	4.48	4.45	4.41	4.38	4.35	4.31	4.27	4.25	4.22
90	4.20	4.17	4.14	4.12	4.09	4.07	4.04	4.02	4.00	3.97

Table 1 was used for evaluating the loss in potential energy of the pendulum during all subsequent investigations made on the first compression of a teased sample.

The agreement with the loss of potential energy calculated for each of the sixty-two determinations by means of equation (1) may be judged from Table 2. In Figure 3 the full line represents Table 1, while the circles represent the individual calculations.

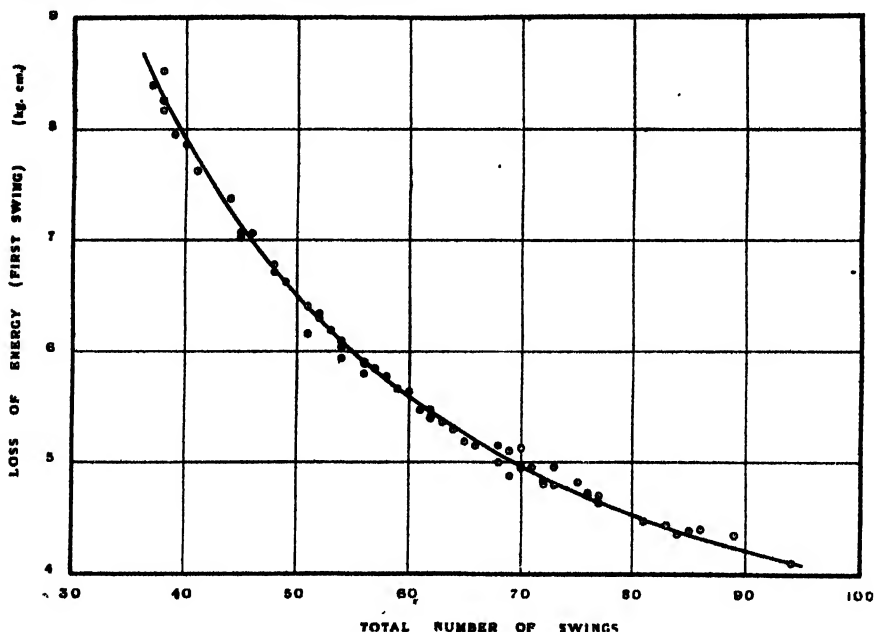


FIGURE 3.—The relation between the loss in potential energy of the pendulum during the first swing and the total number of swings between the two fixed amplitudes.

The differences in Table 2 vary in absolute magnitude from zero to 0.24, and can be attributed to several causes. Part may be due to errors in the observation of the amplitudes, and part to differences in the ability of different wools to recover sufficiently rapidly from compression. Another source of error is no doubt the fact that N , the number of swings, was recorded in complete swings, and according to Table 1, one swing meant a difference of from 0.02 to 0.20 Kg. cm. Thus, for example, one sample might just have been able to record the fortieth swing on the counter, while another may just have failed to record the forty-first. According to Table 1, both samples would have been regarded as having caused a loss of potential energy of 7.90 Kg. cm., while actually a true difference in the number of swings may have been 0.8 of a swing, representing a difference of 0.13 Kg. cm. In routine practice, errors due to this cause were reduced by taking for N the average of at least five determinations.

(v) *The natural damping of the pendulum.*

The values given in Table 1 represent the total loss of potential energy of the pendulum during the first swing. In order to obtain that portion which was due to the work done in compressing the wool, a correction had to be applied for the natural damping of the pendulum.

TABLE 2.

The loss of potential energy of the pendulum (in Kg. cm.) during the first swing as calculated from equation (1) for each of the sixty-two determinations, compared with the values given by Table 1.

N.	Loss of Energy from Equation (1).	Loss of Energy (from Table 1).	Difference.	N.	Loss of Energy from Equation (1).	Loss of Energy (from Table 1).	Difference.
37	8.39	8.48	- 0.09	62	5.42	5.44	- 0.02
38	8.52	8.28	+ 0.24	62	5.47	5.44	+ 0.03
38	8.26	8.28	- 0.02	62	5.41	5.44	- 0.03
38	8.17	8.28	- 0.11	63	5.36	5.37	- 0.01
39	7.95	8.09	- 0.14	64	5.29	5.31	- 0.02
40	7.86	7.90	- 0.04	65	5.18	5.25	- 0.07
41	7.62	7.73	- 0.11	66	5.15	5.19	- 0.04
44	7.37	7.26	+ 0.11	68	5.15	5.07	+ 0.08
45	7.02	7.12	- 0.10	68	5.00	5.07	- 0.07
45	7.07	7.12	- 0.05	69	4.88	5.02	- 0.14
46	7.06	6.98	+ 0.08	69	5.11	5.02	+ 0.09
48	6.78	6.73	+ 0.05	70	5.12	4.96	+ 0.16
48	6.71	6.73	- 0.02	70	4.95	4.96	- 0.01
49	6.62	6.61	+ 0.01	70	4.97	4.96	+ 0.01
51	6.16	6.39	- 0.23	71	4.96	4.91	+ 0.05
51	6.41	6.39	+ 0.02	72	4.80	4.86	- 0.06
52	6.34	6.28	+ 0.06	72	4.83	4.86	- 0.03
52	6.30	6.28	+ 0.02	73	4.79	4.82	- 0.03
53	6.19	6.18	+ 0.01	73	4.96	4.82	+ 0.14
54	6.04	6.09	- 0.05	75	4.82	4.72	+ 0.10
54	5.94	6.09	- 0.15	76	4.70	4.68	+ 0.02
54	6.10	6.09	+ 0.01	76	4.72	4.68	+ 0.04
56	5.89	5.91	- 0.02	77	4.63	4.64	- 0.01
56	5.91	5.91	0	77	4.70	4.64	+ 0.06
56	5.80	5.91	- 0.11	81	4.46	4.48	- 0.02
57	5.85	5.82	+ 0.03	83	4.43	4.41	+ 0.02
58	5.77	5.74	+ 0.03	84	4.35	4.38	- 0.03
59	5.66	5.66	0	85	4.38	4.35	+ 0.03
60	5.64	5.59	+ 0.05	86	4.39	4.31	+ 0.08
61	5.47	5.52	- 0.05	89	4.34	4.22	+ 0.12
62	5.40	5.44	- 0.04	94	4.09	4.09	0

Mean difference..... = - 0.003 Kg. cm.

Standard deviation of differences..... = 0.080 Kg. cm.

By observing successive amplitudes as before, the loss of potential energy with the compression compartment empty was determined with the release stop K (Figure 1) set at six different positions corresponding to different degrees of compression. The mean of ten independent determinations at each position is given in Table 3.

The loss increased as the instrument was set for lower degrees of compression, as a result of increased extension of the springs F (Figure 1), and increased friction of the lever D against the stop K. Since no tension acted on the piston while the results of Table 3 were obtained, a further study was made of the motion of the pendulum while extending a spiral spring attached between the balancing nuts M and the base of the instrument.

TABLE 3.

The loss of potential energy of the pendulum during the first swing, with the compression compartment empty.

	Loss of Potential Energy during first swing.
Compression from 103.3 c.c. to—	Kg. cm.
55 c.c.....	3.11
61 c.c.....	3.15
67 c.c.....	3.18
73 c.c.....	3.21
79 c.c.....	3.25
86 c.c.....	3.28

From the load-extension curve of the spring, the work done in extending it could be determined, and from the initial length and the length at maximum extension, that portion of the loss of potential energy which was due to extension of the springs could be determined. The reduction in amplitude was observed as before, and the total loss in potential energy of the pendulum calculated by fitting equation (1). (The total loss could not be evaluated from the number of swings, as given in Table 1, since Table 1 was applicable only to the case of wool samples which offered a diminishing resistance to compression).

Each determination was repeated five times. Three springs of mean strengths of 63.5 gm./cm., 45.4 gm./cm., and 27.0 gm./cm. respectively were used, and each was stretched by six different amounts. The results are given in Table 4, where E is the total loss in potential energy of the pendulum, e is the loss with the compression compartment empty, as given in Table 3, and W is the work done in extending the springs, as calculated from the load-extension curves of the springs.

As shown in Figure 4, a linear relationship existed between W , the work done in extending the springs, and $(E-e)$, the excess of the total loss of potential energy over the loss with the compression compartment empty. The relationship could be expressed by the equation

$$W = 0.674(E - e) - 0.027 \dots \dots \dots (4)$$

obtained by the method of least squares. Theoretically the line should pass through the origin of axes, but a small error is to be expected in view of the fact that no correction was applied for the work done in raising and lowering the springs, and in slightly displacing the connecting rods, as the magnitude of these factors appeared rather doubtful. The true value of the work done may possibly be given by the right hand side of equation (4) with the second term omitted. In the present investigation the equation as given was, however, employed, and the possible resulting discrepancy may be regarded as extremely small.

It appears that of the total loss of potential energy of the pendulum, just over 3 Kg. cm. was due to friction and air resistance, while of the remainder, two-thirds was due to extension of the springs. The remaining one-third must, therefore, be accounted for by an increase in the natural damping as a result of pressure on the piston. In addition, play of the levers with pressure on the piston may result in a slight further extension of the springs F (Figure 1).

TABLE 4.

The potential energy lost by the pendulum, compared with the work done in extending the springs.

Spring.	Extension (cm.).	Work done (<i>W</i>)	<i>E</i> (Kg. cm.).	<i>e</i> (Kg. cm.).	<i>E-e</i> (Kg. cm.).
1.....	3.8	2.14	6.29	3.11	3.18
	3.4	1.86	5.95	3.15	2.80
	3.0	1.60	5.63	3.18	2.45
	2.7	1.40	5.32	3.21	2.11
	2.3	1.16	5.02	3.25	1.77
	2.0	1.00	4.79	3.28	1.51
2.....	3.8	1.70	5.66	3.11	2.55
	3.4	1.49	5.42	3.15	2.27
	3.1	1.34	5.20	3.18	2.02
	2.7	1.14	4.93	3.21	1.72
	2.2	0.90	4.58	3.25	1.53
	1.8	0.72	4.38	3.28	1.10
3.....	3.8	1.34	5.16	3.11	2.05
	3.4	1.18	4.98	3.15	1.83
	3.1	1.06	4.81	3.18	1.63
	2.8	0.94	4.66	3.21	1.45
	2.5	0.84	4.52	3.25	1.27
	1.6	0.52	4.10	3.28	0.82

E = total loss of potential energy of the pendulum.

e = loss of P.E. with the compression compartment empty.

W = work done in extending springs.

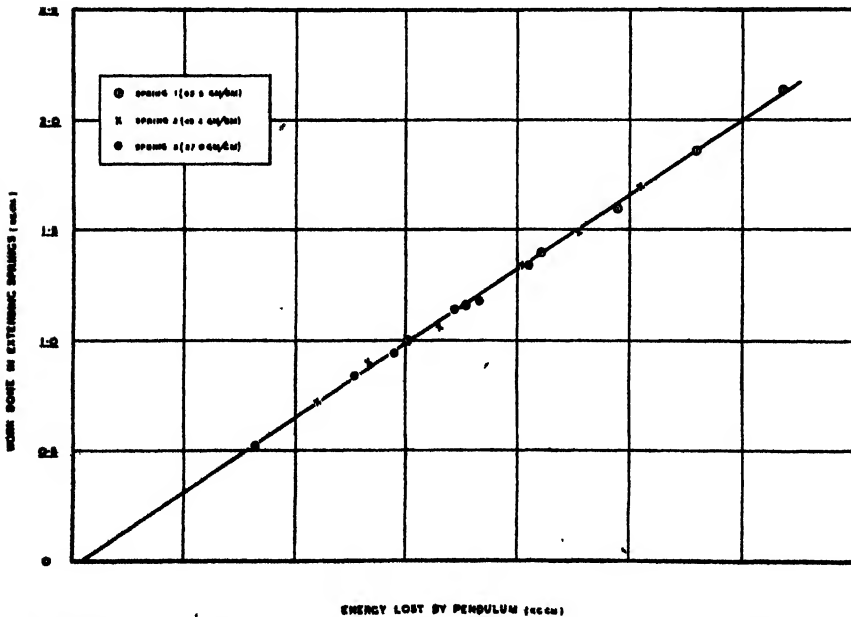


FIGURE 4.—The relation between the work done in extending the springs and the loss in potential energy of the pendulum.

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Since the correction was found to be directly related to the *work done* in extending the springs, the relation as found was assumed to hold for the compression of wool samples, although it is to be noted that the pressure-volume relation of wool is totally different from the load-extension relation of the springs.

(vi) *Air resistance within the compression compartment.*

Besides offering a diminishing resistance to compression, wool differs in another important respect from a spring. While a spring is being extended, the compression compartment is empty, and the enclosed air is expelled through the space between the piston and the walls of the compartment. With five gm. of wool, however, the air has in addition to be expelled through the spaces between the fibres, and the surface area of five gm. of wool of 20μ diameter is approximately 8×10^3 sq. cm. The more rapid the compression, therefore, the greater should be the work done by the pendulum, if the expulsion of the air offers any appreciable resistance, and since the rate of compression is dependent on the amplitude, it is to be expected that the work done should increase with the amplitude of the pendulum.

The point was investigated by starting the pendulum in succession at 48° , 45° , 42° , 39° and 36° , while compressing three wools of high, medium and low compressibility respectively. As before, the amplitudes after successive swings were noted, and the loss of potential energy of the pendulum calculated by fitting equation (1) to the observations. Each determination was carried out four times, and after the necessary corrections had been applied, the results shown in Table 5 were obtained.

TABLE 5.

The effect of the amplitude on the work done (in Kg. cm.) in compressing wool samples.

Sample.	INITIAL AMPLITUDE.				
	48°	45°	42°	39°	36°
1.....	4.06 3.63 3.86 3.78	3.87 3.73 3.89 3.78	3.85 3.70 3.79 3.66	3.68 3.83 3.78 3.77	3.95 3.75 3.83 3.75
Mean.....	3.83	3.82	3.75	3.77	3.82
2.....	2.28 2.10 2.21 2.24	2.24 2.17 2.26 2.17	2.27 2.19 2.32 2.20	2.11 2.24 2.26 2.15	2.09 2.32 2.27 2.19
Mean.....	2.21	2.21	2.25	2.19	2.22
3.....	1.50 1.52 1.48 1.38	1.37 1.46 1.37 1.29	1.37 1.41 1.43 1.34	1.37 1.40 1.40 1.38	1.39 1.36 1.38 1.30
Mean.....	1.47	1.37	1.39	1.34	1.36

In Table 6 the variation between the amplitudes is compared with the variation between the values within each amplitude, by means of the standard deviation.

TABLE 6.
Analysis of variance.

Variance.	D.F.	STANDARD DEVIATION.		
		Sample 1.	Sample 2.	Sample 3.
Between amplitudes.....	4	0.0738	0.0401	0.0875
Within amplitudes.....	15	0.1077	0.0737	0.0493
$z = 0.574.$				

According to Table 6, the variation between amplitudes is less than that within amplitudes in the case of samples 1 and 2, and the variation between the amplitudes may, therefore, be directly attributed to the variation among individual determinations. In the case of sample 3, the variation between amplitudes exceeds that within amplitudes, the value of z (i.e., the natural logarithm of the ratio of the two standard deviations) being 0.574. This value suggests significance at the 5 per cent. probability level, and an examination of Table 5 shows that this is due to the high value at 48° . Now the velocity of the pendulum when started at 48° is approximately $\frac{1}{2}$, that when started at 36° , and as there is no tendency for the work done to alter in the same ratio, it can be concluded that the effect of air resistance in the compression compartment is negligible over the range of velocities examined. This conclusion is confirmed by experiments carried out later on the rate of flow of air through a plug of wool fibres, in an investigation of a method developed by Cassie (1942) for determining fibre diameter. The resistance offered by wool to the passage of air at a density of 10 c.c. per gm. (the highest density employed in the present study) is negligibly small compared to the resistance offered by a sample to compression.

As for the effect of the rate of compression on the resistance of the wool, this must also be considered negligible over the range examined, and the results obtained may be described as those applying under conditions of rapid loading as opposed to those obtained by static methods.

Table 6 shows that the standard deviation of the observations increases as the resistance to compression increases. The average standard deviation of the three samples is 0.081 Kg. cm., in good agreement with the standard deviation of the errors found in Table 2, viz., 0.080 Kg. cm.

(c) *Additional cylinder and piston method employed.*

While the present study has been based on results obtained with the "Pendultex" instrument, certain aspects of the elastic behaviour of wool were investigated by a method giving pressures directly.

For this purpose the simple apparatus illustrated in Figure 5 was constructed. A thick-walled glass cylinder A was let into a base B and closed at the lower end by a steel disc. The base B was firmly attached to a bench with the cylinder projecting over the edge. A steel disc C acted as the piston

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for compressing the wool in the cylinder, and pressure was applied by placing slotted 100 gm. weights *W* on a platform carried by a stiff bronze wire attached to the piston and passing through the wool along the axis of the cylinder.

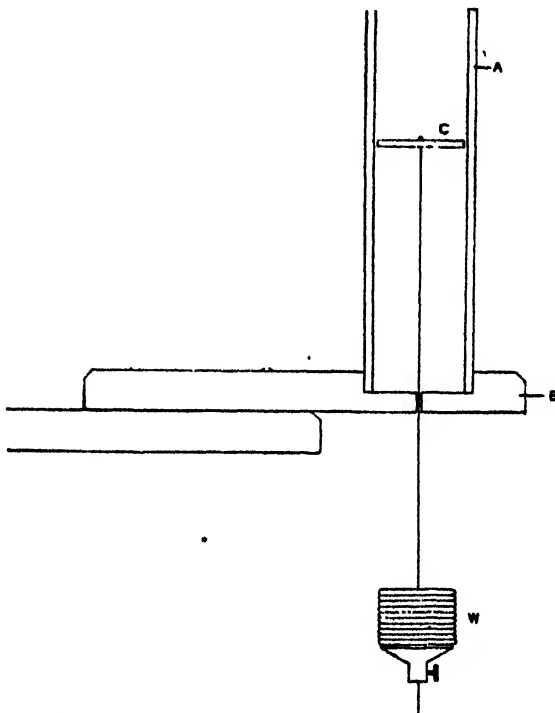


FIGURE 5.—Static cylinder and piston method employed.

For determining the volume of wool at any pressure, the height from the top of the piston to the top of the cylinder was measured at four points situated at the ends of two diameters at right angles to each other. From the area of cross-section of the cylinder and its total length, the volume occupied by the wool could be calculated. The necessary corrections were applied for the thickness of the piston and the variable volume of the wire.

The piston was so attached to the wire that it was capable of a swivel action. This gave an indication of the uniformity of packing of the wool in the cylinder, and determinations were made only when the piston lay horizontal and showed no tendency to slip sideways and bear against the walls of the cylinder. Some such method for indicating the uniformity of packing of the wool is highly desirable, whatever method is employed.

After each weight had been placed on the platform, it was found necessary to tap the base of the instrument fairly vigorously for about five minutes before the volume became constant. Previous investigators, employing other static methods, have noted the same lag in taking up the final volume. It is probably due to friction of the fibres against the walls of the containing vessel, and of the fibres among themselves.

B. THE ELASTIC BEHAVIOUR OF WOOL IN BULK.

(a) *Historical.*

It has already been stated that previous investigators have found that when wool samples are taken through successive cycles of compression and release, the position of the pressure-volume curve is altered, at first rapidly and then more slowly until the curve appears to reach a constant position. This was found to be the case when the wool was compressed in the apparatus shown in Figure 5, and was shown in the case of the "Pendultex" instrument by an increase in the number of swings recorded.

Moreover, at any value of the pressure, the volume occupied during removal of the compressing force was lower than that during its application, but when the position of the curve became constant, the sample returned to its original volume on complete removal of the pressure.

While some investigators were content to draw their conclusions from the plotted curves, others measured the work done during compression and release, while two obtained a relation between pressure and volume.

M. and J. Eggert (1925) used the relation

$$\phi^\gamma (\pi + \pi_0) = \pi_0 \cdot 10^\gamma$$

where π was the pressure and ϕ ten times the ratio of the volume to the volume at zero applied pressure. The constant π_0 was regarded as a measure of the softness (Weichheit) of the wool, while γ was taken to indicate the pliability (Geschmeidigkeit).

Schofield (1938) stated that for the later points, the equation

$$\text{Pile thickness} = 12.9 (\text{Load})^{-0.3}$$

very nearly fitted his experimental results.

(b) *The relation between pressure and volume.*

The Eggert equation, together with Schofield's results, suggested that the pressure should bear a linear relation to the inverse cube of the volume. The author accordingly plotted pressure as a function of the inverse cube of the volume for data given by Pidgeon and van Winsen (1934), Larose (1934) and Schofield (1938). Except for the points representing small degrees of compression, the relations were found to be linear in all cases. Typical examples are illustrated in Figure 6, where the units have been plotted arbitrarily, since the different authors used different units. It is to be noted that the curves shown were obtained by three different methods, with wool under widely different conditions.

With the cylinder and piston method illustrated in Figure 5, it was found that initially the pressure varied nearly as the inverse first power of the volume, the index increasing (negatively) with successive cycles until during the final constant cycle, the index became -3 , in complete agreement with the curves illustrated in Figure 6. Observation of the wool during compression showed that initially most of the reduction in volume was taken up by that portion of the wool which was nearest to the piston, and the sample could then be regarded as behaving like a spring. With increasing pressure more and more of the wool was compressed, until the whole mass showed the same density of packing.

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On the release of the pressure, it was evident that a certain amount of inter-locking of the fibres had taken place, and the friction against the walls of the cylinder prevented that portion of the wool furthest removed from the piston from opening up completely. The result was that the sample did not reach its initial volume, and the volumes at the same pressures during the following cycle of compression were considerably lower than those during the first cycle. This process was repeated during successive cycles, the reduction in initial volume becoming smaller with each cycle until the initial volume became constant. Even at this stage, however, it could be clearly observed that on removal of the pressure, the wool nearest to the piston opened up to a greater extent than that portion furthest removed from the piston.

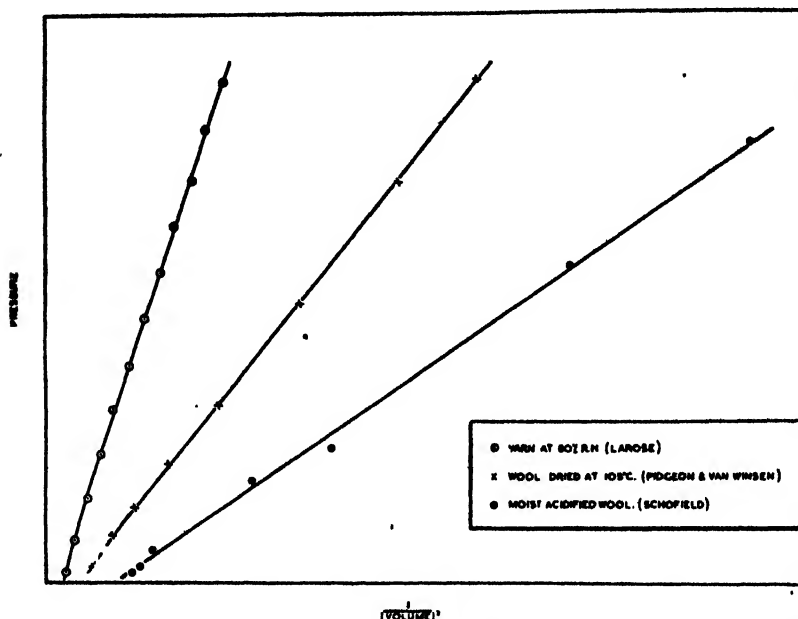


FIGURE 6.—The pressure as a function of the inverse cube of the volume for data given by Schofield (1938), Pidgeon and van Winsen (1934) and Larose (1934). (Arbitrary units and origin.)

The results of the observations on the wool during compression may be summed up as follows:—

1. The compression during the initial cycle is not uniform, since the density of packing is not uniform throughout the sample.
2. The curves obtained from successive cycles exhibit a tendency to coincide at high pressures.
3. In the final constant cycle, the results obtained at low pressures should not be considered together with those obtained at higher pressures, since the density of packing the wool is not uniform when the compressive force is completely removed, and the subsequent compression resembles that of a spring initially.
4. In the final constant cycle, the relation between pressure and volume is such that the pressure bears a linear relation to the inverse cube of the volume.

(i) *The inverse cube equation.*

The relation between pressure and volume may therefore be expressed by the equation

$$p = A \left(\frac{1}{v^3} - \frac{1}{v_0^3} \right) \dots \dots \dots (5)$$

where p is the pressure at volume v , v_0 is the volume at zero applied pressure and A is a constant. Equation (5) is that of the Eggerts with $\gamma=3$.

Hence the work done in compressing a sample from a volume v_1 to a volume v should be given by

$$W = \frac{A}{2} \left(\frac{1}{v^2} - \frac{1}{v_1^2} \right) + \frac{A}{v_0^3} (v - v_1) \dots \dots \dots (6)$$

With the "Pendultex" instrument the relation was studied by selecting 5 gm. samples of Merino wools, A, B, C and D and one sample of Romney wool, E. The samples were compressed to various volumes and the work done calculated from the number of swings recorded. The means of five determinations each are given in Table 7, and illustrated in Figure 7.

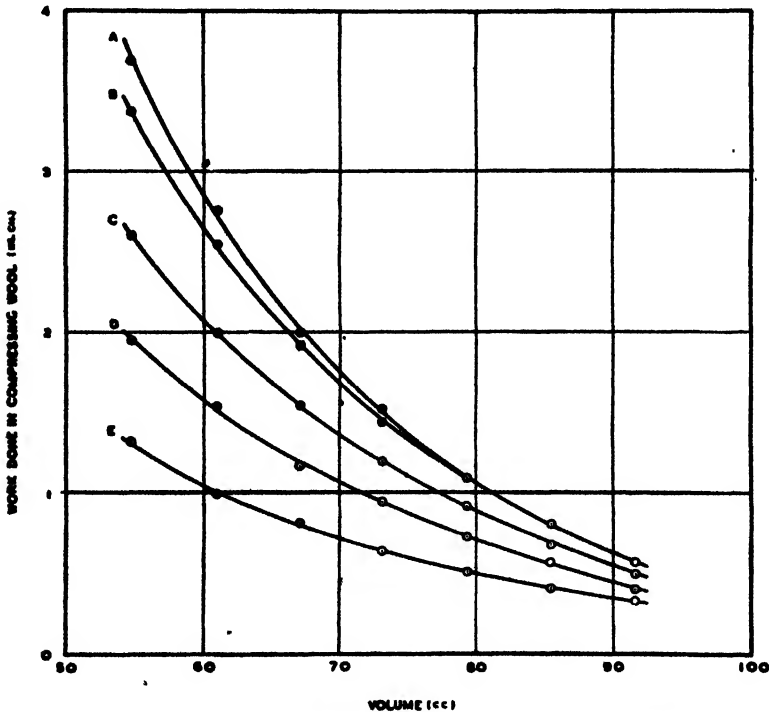


FIGURE 7.—The work done in compressing 5 gm. of different wools by the dynamic method from 103.3 c.c. to various volumes, as a function of the volume.

The result of applying equation (6) to the data of Table 7 was not satisfactory. It was obvious that the wool did not behave as if it was being compressed from the volume v_1 , and when v_1 was derived from the experimental results the coefficient of the second term was positive in some cases and

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negative in others. As in the case of wool compressed by static methods, small compressions were not transmitted uniformly throughout the bulk of the material owing to the frictional forces in operation. The effect was greater in the case of the "Pendultex" instrument, owing to the short duration of the compression.

TABLE 7.

The work done (in Kg. cm.) in compressing 5 gm. of different wools from 103.3 c.c. to various volumes in the "Pendultex" instrument.

Final Volume. (c.c.).	SAMPLE.				
	A.	B.	C.	D.	E.
54.8.....	3.69	3.37	2.60	1.95	1.32
61.0.....	2.76	2.55	1.98	1.54	1.00
67.1.....	2.00	1.92	1.55	1.17	0.82
73.2.....	1.52	1.44	1.20	0.95	0.64
79.4.....	1.09	1.09	0.92	0.73	0.51
85.5.....	0.81	0.81	0.68	0.57	0.41
91.7.....	0.57	0.58	0.50	0.40	0.33

Approximation.—In Figure 8 the work done is plotted as a function of the inverse square of the volume. It is evident that for compressions to volumes below about 75 c.c., the relation becomes linear, showing that the wool is being compressed practically uniformly, and the term containing $\frac{1}{v^2}$ in equation 6 becomes predominant.

The samples designated A and E represented very nearly the extremes in compressibility found in the present investigation, hence it was assumed that the equation

$$W = \frac{a_1}{v^2} - a_2 \dots \dots \dots (7)$$

where v was the final volume, was applicable to all 5 gm. samples tested, when compressed to volumes below 75 c.c. Applying equation (7) to the five samples under consideration, and neglecting volumes above 75 c.c., the constants shown in Table 8 were obtained.

TABLE 8.

The constants a_1 and a_2 of equation (7) evaluated for the five wools, A, B, C, D and E compressed to various volumes (see Table 7).

Sample.	a_1	a_2	a_2 (calculated from Equation 8).	Difference.
A.....	14,970	1.289	1.292	+ 0.003
B.....	13,200	0.989	0.981	— 0.008
C.....	9,529	0.575	0.580	+ 0.005
D.....	6,927	0.347	0.351	+ 0.004
E.....	4,578	0.211	0.207	— 0.004

As shown by Figure 9, a simple relation held between the constants a_1 and a_2 of equation (7), viz.,

$$a_2 = 5.344 \times 10^{-9} \cdot a_1^2 + 0.0947 \dots \dots \dots (8)$$

The values of a_2 calculated from equation (8) are given in Table 8.

Combining (7) and (8), and writing a for a_1 , the work done in compressing a five gm. sample to a volume v becomes

$$W = \frac{a}{v^2} - 5.344 \times 10^{-9} \cdot a^2 - 0.0947 \dots \dots \dots (9)$$

Equation (9) contains one constant, a , which is readily calculated for any values of W and v . The relation (8) between a_1 and a_2 is an approximation, but it points to some connection between a_1 and v_0 .

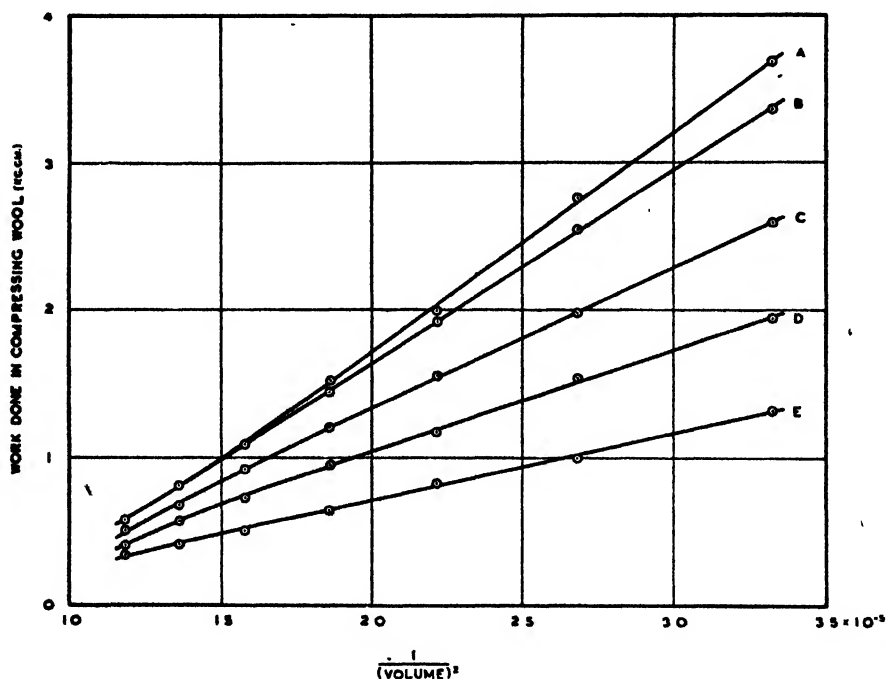


FIGURE 8.—The work done in compressing 5 gm. of different wools by the dynamic method from 103.3 c.c. to various volumes, as a function of the inverse square of the volume.

Accuracy of the approximation.—The extent to which it is possible to fit equation (7) to results which in reality must be regarded as following equation (6) may be judged from the case of sample A. The results of this sample when applied to equation (6) yield the equation

$$W = \frac{15943}{v^2} + 0.007491 \cdot v - 2.016 \dots \dots \dots (10)$$

Values of W calculated from equation (10) at equal intervals of $\frac{1}{v^2}$ are given in Table 9.

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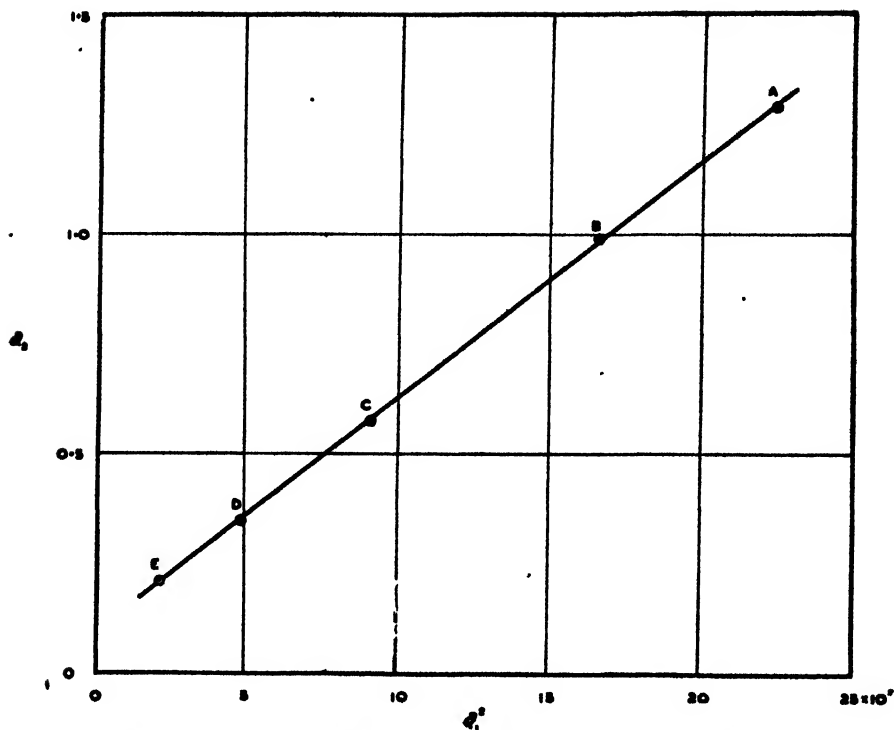


FIGURE 9—The relation between the constants a_1 and a_2 of equation 7.

TABLE 9.

The work done as calculated from equation (10) for equal intervals of the inverse square of the volume.

v (c.c.).	$\frac{1}{v^2}$	W (Kg. cm.).	First Difference. (Kg. cm.).
100.0	0.00010	0.33	0.25
91.3	12	0.58	0.27
84.5	14	0.85	0.27
77.5	16	1.12	0.29
74.5	18	1.41	0.29
70.7	20	1.70	0.30
67.4	22	2.00	0.29
64.6	24	2.29	0.30
62.0	26	2.59	0.31
59.8	28	2.90	0.30
57.7	30	3.20	0.30
55.9	32	3.50	0.31
54.2	34	3.81	

For compression to volumes below 75 c.c., the first differences are very nearly constant, and a linear relation between work done and the inverse square of the volume may be assumed for purposes of calculation.

Regarding the constant a as calculated from equation (9) as an approximation to the constant $\frac{A}{2}$ of equation (6), the relation between them may be obtained by differentiating both expressions for W with respect to $\frac{1}{v^2}$, and equating the results. Thus—

$$a = \frac{A}{2} \left(1 - \frac{v^3}{v_0^3}\right)$$

The value of v_0 , the volume of a 5 gm. sample at zero pressure, was estimated to be in the region of 160 c.c., by observing what portion of a 5 gm. sample appeared to fill the compression compartment completely without applied pressure. Equation (9) was based on values of v between 73.2 and 54.8 cc.. For these limits the factor $\left(1 - \frac{v^3}{v_0^3}\right)$ has the values 0.904 and 0.960 respectively. The mean value of 0.932 suggests that the approximation gives the value of $\frac{A}{2}$ too low by about 7 per cent.

Similarly equation (10) with $\frac{A}{2} = 15943$ gives the pressure at 55 c.c. as 184 gm./sq. cm., while equation (9) with $a = 14970$ gives 170 gm./sq. cm., a difference of 7 per cent.

The differences between the observed values given in Table 7 and those calculated after fitting equation (7) have a standard deviation of 0.018 Kg. cm., which is far below those found for the errors in Tables 2 and 6, viz., 0.080 Kg. cm. The use of Table 1, after each observation had been repeated five times, consequently reduced the error considerably.

It has been shown that the results of compressibility determinations agree with the assumption that the pressure varies in a linear manner with the inverse cube of the volume. The further refinement of reducing the volume by a quantity v' , the volume of wool substance, may be added, on the basis that the limiting value of the volume will theoretically be the volume of the wool substance itself, although such a condition may not be attainable in practice. The equation

$$p = A \left(\frac{1}{(v - v')^3} - \frac{1}{(v_0 - v')^3} \right) \dots \dots \dots (5a)$$

is then obtained. As regards agreement with experimental data there appeared to be little to choose between the two equations (5) and (5a), but for the comparison of different wools it is essential that either one or the other be adhered to.

(ii) *Exponential equation.*

A large number of empirical relations have been investigated, and an equation which provides an excellent fit will be considered in some detail, since it exhibits some interesting features.

Equations of the class

$$p = P_0 Q \left(\frac{m}{v - v'} \right)^n - R$$

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where m is the mass, and P , Q and R , are constants, fit the observational results over a range of several values of n . For simplicity the case $n=1$ has been considered, giving the equation

$$p = Pe^{\frac{Qm}{(v-v')}} - R \dots \dots \dots (11)$$

Equation (11) gives a better fit than equation (5), owing to the fact that it contains three unknown constants. As will appear later, the accuracy of compressibility measurements does not justify the use of an equation containing more than two constants, and erroneous conclusions may therefore be drawn from the constants of equation (11). In this connection it is to be noted that in the present study the quantity v_0 has in no case been adopted as an observed constant in an equation, since it has been affirmed that results at higher pressures should not be considered together with those obtained at low pressures. Equation (5) is, therefore, regarded as a two-constant equation which may be written as

$$p = \frac{A}{v^3} - B \dots \dots \dots (5b)$$

Similarly equation (11) is regarded as containing three unknown constants, although the constant R is made up of the constants P , Q and v_0 .

Applying equation (11) to the pressure-volume results of five wools as obtained by the static method, the values for the constants P , Q and R shown in Table 10 were obtained. (In fitting the equation, by the method given in Appendix A, p was taken as the independent variable, as this quantity was measured with negligible error, and the quantity $\frac{m}{(v-v')}$ as the dependent variable.

TABLE 10.

The constants P , Q and R obtained by fitting equation (11) to the results of compressing five samples by the static method.

Sample.	P	Q	R
1.....	179	20.0	301
2.....	447	17.1	798
3.....	526	16.8	1,048
4.....	473	21.2	1,060
5.....	613	20.3	1,270

The five samples are given in increasing order of resistance to compression, as given by the position of the pressure-volume curves and the coefficient A of equation (5). The results of Table 10 suggest that the constant Q may be independent of the sample, and that the variation found is due to experimental error.

The possibility must be considered, however, that the constancy of Q may be due to the fact that the results actually follow equation (5). A direct comparison of the constants of the two equations is made impracticable by

the non-integrability of the function $e^{\frac{1}{v}}$ in finite terms. Accordingly the best-fitting values of the constants of equation (5) were calculated from the results as employed for the evaluation of the constants of equation (11) given in Table 10. From the five equations so obtained, values of $\frac{m}{(v - v')}$ for the same values of p as the experimental observations were calculated. Equation (11) was then fitted to the values so obtained. The values of P , Q and R given in Table 11 are therefore those obtained by fitting equation 11 to data which rigorously follow equation (5).

TABLE 11.

The constants P , Q and R obtained when equation (11) is fitted to results which rigorously follow equation (5).

Sample.	P	Q	R
1.....	247	17.9	453
2.....	230	22.0	436
3.....	240	22.6	492
4.....	202	26.4	646
5.....	249	28.6	596

According to Table 11 the coefficient Q increases with the resistance to compression, and the supposed constancy of this coefficient in Table 10 cannot be attributed to the fact that the results follow equation (5). The coefficient P shows a tendency to be constant.

It appears reasonable to conclude that the coefficient Q is constant and independent of the sample. Taking the value 20 for Q , the values of P and R as calculated from the experimental observations are given in Table 12. The coefficient A of equation (5) is included for comparison.

TABLE 12.

The values of P and R on the assumption that Q has the value 20 in all cases.

Sample.	P	Q	R	A	$\frac{A}{P}$
1.....	177.2	20	297.1	1.198×10^6	6.76×10^3
2.....	300.8	20	536.4	1.971	6.55
3.....	337.1	20	712.3	2.221	6.59
4.....	645.0	20	1,186.3	3.702	6.79
5.....	631.6	20	1,303.6	4.429	7.01

When Q is given the constant value 20, the coefficient P is practically proportional to the coefficient A of equation (5) as appears from the last column of Table 12. Either of the two coefficients may therefore be regarded as suitable for expressing the compressibility of a sample. This is

not the case for the coefficient P as given in Table 10, showing that a three-constant equation is not to be justified on practical grounds. Taking a fixed value for Q reduces the number of unknown constants to two.

The next point to be considered is how the value 20 for Q compares with that calculated from the results of previous investigators. The available data are those of Larose (1934) and Pidgeon and van Winsen (1934), and the coefficient Q as calculated from their results by the present author is given in Table 13.

TABLE 13.

The coefficient Q calculated from the results of previous investigators.

Author.	Sample.	Mass of Sample.	Q
		Gm.	
Larose (1934).....	Yarns at 50 per cent. relative humidity.	3.22	
	No. 1 Undyed.....		17.0
	No. 1 Dyed.....		16.5
	No. 2 Undyed.....		12.3
	No. 2 Dyed.....		12.5
Larose (1934).....	Yarns at 60 per cent. relative humidity.	3.25	—
	No. 1 Undyed.....		12.2
	No. 1 Dyed.....		17.5
	No. 2 Undyed.....		9.8
	No. 2 Dyed.....		7.7
Larose (1934).....	Yarn (different weights).....	2.20	13.1
		3.26	13.6
		4.34	13.5
Pidgeon and van Winsen (1934)	Loose wool—		
	Dry.....	3.5	12.8
	95 per cent. relative humidity.....	5.0	13.9
	Mean.....		13.2

The results of other workers tend to give considerably lower values than those obtained in the present investigation, and it is of interest to consider the possible factors which may influence the value of the coefficient Q .

1. *State of the sample.*—Sample 3 was a short-stapled wool, and after washing, the staples were found to have formed small compact lumps. A determination on the sample in this form gave the value 23.0 for Q , while after the lumps had been carefully removed, the value 16.8 was obtained. The lumps, therefore, caused an increase in the value of Q , and it may be supposed that the yarn form would do the same, but the values calculated from Larose's results on yarn are lower than those obtained here.

2. *Successive cycles.*—The values obtained for successive cycles are given in Table 14.

While the experimental error is larger, since each value except the last is based on one determination only, the trend is unmistakable. The value of Q increases with successive cycles of compression. This result does not, however, explain why the results of previous authors are lower, since Larose proceeded to the fifth or sixth cycle, while Pidgeon and van Winsen's values

are based on the fourth cycle. These authors considered that the wool had by then attained a final steady condition, though it is to be noted that in the present investigation constancy was obtained at from the ninth to the sixteenth cycle.

TABLE 14.

The coefficient Q obtained for successive cycles of compression.

Cycle No.	Sample 1.	Sample 4.
1.....	13.7	9.0
3.....	—	9.9
5.....	16.9	17.4
7.....	21.5	18.4
Final.....	20.0	21.2

3. *Rate of loading.*—An uncontrolled factor which may influence the value of Q is the rate of loading. While the static method employed in the present study did not permit of the accurate control of the rate of loading, initial experiments in which determinations were carried out more rapidly gave the lower value of 15 for Q in the case of sample 1. The determinations from which the results of Table 10 were obtained, were performed extremely slowly with vigorous tapping of the base of the instrument, and approximately five minutes were allowed to elapse before a reading of the volume was taken at each pressure. In this way frictional effects were to some extent overcome, and allowance was made for a lag due to any other cause.

Application to dynamic method.—The pendulum method gives the work done in compressing a sample, so that the application of equation (11) involves the integration of the function

$$\frac{Qm}{e(v - v')}$$

with respect to v , and this can only be done by means of an infinite series. The small number of observations also precludes the accurate evaluation of the coefficient Q . The best fitting value of Q is not, however, a critical one, as judged by the closeness of fit of the equation when both the values 20.0 and 13.3 are assumed for Q . When the value 13.2 is assumed, the ratio of the constants R and P is approximately the same for the five samples, with a mean value of 1.725. Taking $Q = 13.2$ and $\frac{R}{P} = 1.725$, the ratio of the coefficient a of equation (9) to the coefficient P of equation (11) is exactly the same for the five wools, viz., 1.65×10^5 . It is evident that for comparing different wools, it is immaterial which of the two equations is employed, provided the coefficient Q is assumed to be the same for all wools. This conclusion is confirmed by Table 12.

The exponential equation provides an interesting field of investigation. Its further study was, however, considered to fall outside the scope of the present investigation. Assuming the equation to fit experimental observations, the work done in compressing a sample may be evaluated by means of the tables in Appendix B.

(c) The relation between the work done and the mass of the sample.

Larose (1934) compressed successively 2.20 gm., 3.26 gm., and 4.34 gm. of the same yarn and found that the ratio of mass to volume at the same pressure was constant. The same result was obtained in the present study by the static method, and it was taken for granted in the employment of equation (11). Assuming the pressure to be a linear function of the inverse cube of the volume, the pressure and work done may be expected to bear a linear relationship to the cube of the mass of the sample.

For comparative purposes it would be sufficient to specify that a certain mass (5 gm. in the present study), be used for a determination. Cases occur, however, where a smaller quantity only is available, and in any case it is far more rapid and convenient to weigh out approximately 5 gm. The relation between the work done, as determined by the dynamic method, and the mass of the sample was, therefore, investigated.

Owing to the variation within a sample, a careful system of sampling had to be employed. Accordingly 6 gm. of two samples of high and low compressibility were weighed out. Weights from 3 gm. to 6 gm. at intervals of 0.5 gm. were allotted the numbers 1 to 7, and placed five times in random order by means of tables of random numbers. The various weights were then compressed in these orders in the instrument. After each determination the whole sample of 6 gm. was placed together, so that the next weight was selected from the whole sample. This procedure ensured that no bias occurred in the matter of sampling, while the repeated randomisation of the order ensured that any changes produced in the wool as a result of the extensive handling should be distributed throughout the various masses employed.

The results are given in Table 15.

In Figure 10 the work done is plotted as a function of the cube of the mass. Beyond a certain value of the mass the relation is linear, but the straight lines do not pass through the origin of axes. They cut the y -axis at points which differ for the two samples but are independent of the degree to which each sample is compressed.

An approximation similar to that made when the same sample is compressed to different volumes must obviously be made, probably by the exclusion of certain terms from the general equation, and it is necessary to determine how to fit the results obtained into equation (9), and to show how the coefficient a may be calculated when a mass other than 5 gm. is employed.

It is found that if for v in equation (9) a quantity u is substituted, such that

$$\frac{1}{u^3} = \frac{m^3}{5^3} \left(\frac{1}{v^3} - \frac{1}{v_1^3} \right) + \frac{1}{v_1^3} \dots\dots\dots (12)$$

the value of a as given by equation (9) can be calculated for the different masses. The last two columns of Table 15 give the value of $\frac{1}{u^3}$ as calculated from equation (12), and the corresponding value of a as calculated from equation (9). The calculations are seen to be valid in the range 4 to 6 gm., and an adjustment can be made to 5 gm. for any quantity of wool between these limits.

TABLE 15.

The work done in compressing different masses of two samples from 103.3 c.c. to two different volumes.

Sample.	Final Volume (v). c.c.	Mass (m) gm.	Work done. (Kg. cm.).	$\frac{1}{u^2}$	a (Equation 9).
1.....	55.0	3.04	0.88	0.000143	—
		3.58	1.34	178	13.7×10^3
		4.05	1.95	217	14.8
		4.54	2.77	270	15.2
		5.04	3.81	337	15.3
		5.53	4.93	416	14.9
		6.08	6.50	522	14.9
	62.4	3.05	0.59	0.000127	—
		3.60	0.96	152	12.2×10^3
		4.04	1.36	177	14.7
		4.55	1.94	215	15.3
		5.06	2.62	262	14.8
		5.55	3.50	318	15.2
		6.09	4.56	391	15.0
2.....	55.3	3.04	0.59	0.000146	6.0×10^3
		3.59	0.83	180	6.3
		4.05	1.13	218	6.8
		4.56	1.36	270	6.1
		5.06	1.80	336	6.3
		5.55	2.27	413	6.2
		6.03	2.79	502	6.1
	62.1	3.04	0.44	0.000131	5.2×10^3
		3.59	0.64	155	6.0
		4.05	0.86	182	6.5
		4.56	1.05	219	6.2
		5.06	1.40	265	6.5
		5.54	1.72	320	6.4
		6.03	2.06	384	6.1

Besides the fact that an approximation is probably made in assuming linearity between the work done and the cube of the mass of the sample, it must be pointed out that quantities less than about 3 gm. do not fill the compression compartment completely, so that v_1 is not the same for masses above and below 3 gm.

Taking the calculated values of a from 4 to 6 gm., the standard deviation of the differences from the mean is found to be 0.23×10^3 . For compression to various volumes, as given in Table 7, the standard deviation is 0.26×10^3 . The two values are in close agreement, and if a difference between them exists, it may be presumed to be due to the difficulty of determining the volume as accurately as the mass.

(d) *Comparison of results obtained by the static and the dynamic methods.*

It has been shown that the coefficient a as given by equation (9) can be regarded as an approximation to the coefficient $\frac{A}{2}$ of equation (6), and it was

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estimated to be too low by about 7 per cent. It is interesting to see how the coefficient a obtained by the dynamic method agrees with the coefficient $\frac{A}{2}$ as given by the static cylinder and piston method

The result of testing five wools by the two methods is given in Table 16, where the results refer to 5 gm. of material and the pressure is measured in Kg./cm.²

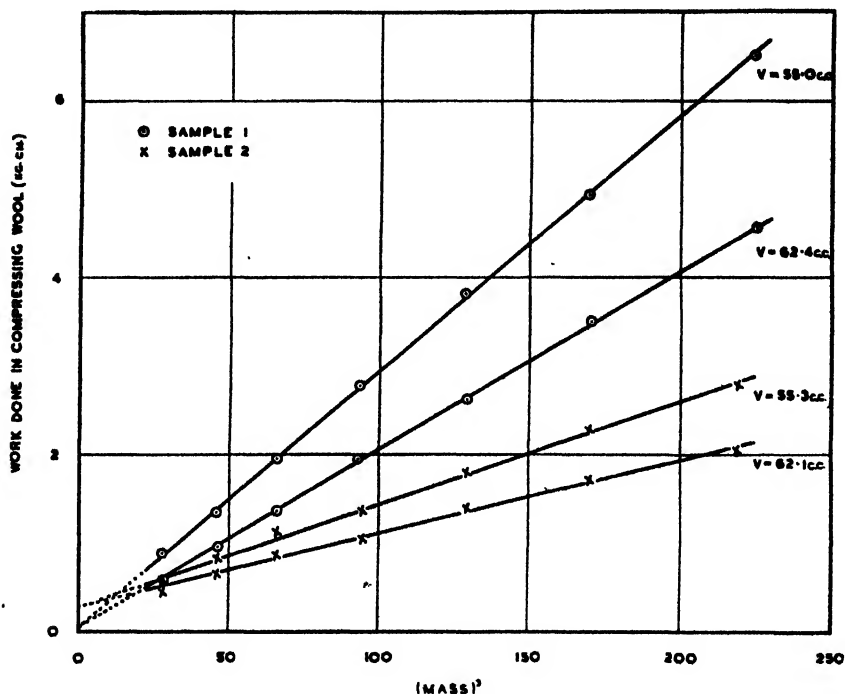


FIGURE 10.—The work done in compressing different masses of wool by the dynamic method, as a function of the cube of the mass.

TABLE 16.

A comparison of the coefficients obtained by the static and dynamic methods.

Sample.	a (Dynamic Method).	$\frac{A}{2}$ (Static Method).	Ratio.	Difference.
1.....	5.8×10^3	3.2×10^3	1.81	2.6×10^3
2.....	9.4	5.2	1.81	4.2
3.....	11.5	6.0	1.92	5.5
4.....	15.0	9.8	1.53	5.2
5.....	15.5	11.7	1.32	3.8

While the order of magnitude is the same in the two cases, it is evident that the coefficients differ in two respects. The coefficient a of the dynamic method is in all cases the greater, and the differences do not follow any law consistently, as shown by the ratios and differences.

Several reasons may be advanced as being responsible for the differences. In the first place, the value of a was obtained from the first compression of a sample, while the coefficient $\frac{A}{2}$ was calculated from the final constant cycle of compression by the static method. The question immediately arises as to whether the coefficients obtained by the initial rapid compression and the final slow compression are comparable. Evidence occurred during the investigation which indicated that with the dynamic method the differences between successive cycles lay, not in the coefficient a itself, but in the constants of equation (9) from which the coefficient a was calculated, since the difference in the work done between the initial and the final compressions bore a linear relationship to a^2 , as calculated from the first compression. The evidence could not be regarded as conclusive, however, and the point was not investigated further, but it is significant that the inverse cube law was found to hold for the first compression by the dynamic method, and only for the final constant cycle of compression by the static method.

In the second place, the difference in the rate of compression must be regarded as one cause of the difference obtained by the two methods. In the static method, the compression was performed extremely slowly, and an attempt was made to overcome the effects of friction as far as possible. The rapidity of compression by the dynamic method, on the other hand, would include frictional effects in the work done during compression. Assuming the friction to be proportional to the pressure, this would result in an increase in the coefficient a .

A third cause, especially of the irregularity of the differences, lies in the state of the sample. The effect of lumpiness on the coefficient Q in the case of sample 3 has already been considered. By making determinations on the sample by both methods before and after removal of the lumps, it was found that the effect of the lumps had been to increase the coefficient $\frac{A}{2}$ (static method) from 6.0×10^3 to 7.9×10^3 , an increase of 32 per cent. The coefficient a (dynamic method) had been increased from 11.5×10^3 to 12.4×10^3 , an increase of 8 per cent. The static method may therefore be regarded as being more sensitive to the state of the sample than the dynamic method. A part of the discrepancy between the two coefficients may be attributed to this cause, for in spite of careful separation of the fibres, a small amount of residual lumpiness seemed unavoidable.

It is of interest to note that the percentage difference between the work done during compression and release by the static method was 55 per cent. for the sample in the lumpy state and 56 per cent. after it had been teased out. The difference was insignificant, in spite of the 32 per cent. increase in the value of the coefficient $\frac{A}{2}$.

(e) The arithmetical expression of compressibility.

The present study has been based on results obtained with the "Pendultex" instrument. It has been shown that when a 5 gm. sample is

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compressed from a volume of 103.3 c.c. to a volume v , where v is less than 75 c.c., the relation between the work done, W , in Kg. cm., and the volume v can be expressed by the equation

$$W = \frac{a}{v^2} - 5.344 \times 10^{-9} \cdot a^2 - 0.0947 \dots \dots \dots (9)$$

for values of a from 4.6×10^3 to 15.0×10^3 .

For the purpose of the present investigation, the coefficient a as defined by equation (9) has been taken as *the coefficient of resistance to compression*. The coefficient is derived from the equation

$$p = A \left(\frac{1}{v^3} - \frac{1}{v_0^3} \right) \dots \dots \dots (5)$$

assumed to represent the relation between pressure and volume, where a is an approximation to the coefficient $\frac{A}{2}$ of equation (5). In the more symmetrical form

$$p = A' \left(\frac{v_0^3}{v^3} - 1 \right) \dots \dots \dots$$

the dimension of A' is that of pressure in Kg./cm.² As the dynamic method does not give the value of v_0 , the latter form could not be adopted, and the dimension of a is, therefore Kg./cm.²(c.c.)³. For convenience the unit will simply be designated Kg. cm.⁷ per 5 gm.

If compressibility is given its usual definition of $\frac{1}{v} \cdot \frac{dv}{dp}$, it follows from equation (5) that it is equal to $-\frac{v^3}{3A}$, and at any volume is thus proportional to the reciprocal of the coefficient A , and hence of a . A high value of a indicates that a sample offers a high resistance to compression, and consequently has a low compressibility.

C. TECHNIQUE EMPLOYED FOR THE MEASUREMENT OF COMPRESSIBILITY.

In the case of a fleece or a similar quantity of wool, the whole bulk was spread out evenly over a table and divided into ten or more zones. Staples were taken at random from each zone in succession until a composite sample of 90 gm. had been collected. The composite sample was divided into two, and from each portion a sub-sample of 15 gm. was made up by removing a few strands from each staple. The remaining 60 gm. was kept for the determination of other properties.

The number of crimps per inch of staple of each of the strands occurring in the two sub-samples was next determined, and a small portion of each of the strands was placed together in a bundle for fibre thickness measurements.

The two sub-samples were then washed in three changes of benzene at 40° C., after which the dust and vegetable matter were removed by teasing out the wool thoroughly. Finally the sub-samples were washed twice in distilled or rain water at 50° C.

The samples were put out to become thoroughly airdry. As a rule the relative humidity was in the region of 50 per cent., but during damp weather the wool was kept until several dry days had passed. When subsequently

placed in a room maintained at 65 per cent. relative humidity, the samples therefore attained equilibrium under adsorption conditions. No attempt was made to dry the wool by heating, as this procedure reduces the affinity of wool for water.

The first set of measurements was made with the room maintained at 70 per cent. relative humidity and 70° F. (21.1° C.) temperature, but the conditions were later changed to 65 per cent. relative humidity and 70° F. temperature. A study was made of the influence of adsorbed water on the resistance to compression, and it was found that results obtained at 70 per cent. relative humidity could be converted to those at 65 per cent. relative humidity by multiplying by the factor 1.12. All results given in the present paper, therefore, refer to 65 per cent. relative humidity and 70° F. (21.1° C) temperature.

The samples had to be conditioned for at least fourteen days before constant results were obtained. At the end of this period 5 gm. was weighed out from each sample on a rough balance, and the resistance to compression determined in the "Pendultex" instrument five times in succession. After each determination the sample was removed from the compartment and teased out into as loose a mass as possible. Since the duplicates were compressed alternately, one sample was teased out while the other was being compressed, and no time was wasted. Finally the samples were weighed correct to 0.01 gm.

From the number of swings, the volume to which the sample had been compressed, and the weight of the sample, the coefficient a as defined was calculated.

In order to ensure that no additional damping forces had developed, the pendulum was set swinging with the compression compartment empty, at regular intervals, and the number of swings noted.

D. DISCUSSION.

(a) *Methods of measuring compressibility.*

The determining factor in the choice of a method will be the nature of the information desired. The balloon method, and cylinder and piston methods such as employed by Larose (1934) and by the author give the compression and release curves separately. Consequently when the two curves have to be compared, for example by the work done during compression and the work done during removal of the compression force, any of these methods should prove suitable.

The author prefers his method of applying the load to that of employing a spring, since it obviates the calibration of the spring and makes for greater stability of the piston. By inverting the system and applying the weights to a cord drawn over a pulley, it should be a simple matter to adapt the method for studying the compressibility of wool immersed in various liquids.

Various authors seem to be agreed that the balloon method is more tedious than other methods, but one of the most serious drawbacks must be the fact that the calculation of each volume depends on the measurement of the volume at one pressure. From the author's own limited experience of the method, it was concluded that the accurate determination of the volume

THE COMPRESSIBILITY OF WOOL.

at one pressure is a matter of some difficulty, and it must be repeated for every test. The error in the measurement is reflected in the calculated value of all other volumes.

Cylinder and piston methods do not suffer from this disability. On the other hand, exception may be taken to these methods on the ground that the wool is compressed from one side only, whereas in the balloon method the compression takes place over the whole surface of the sample in contact with the balloon. This does not, however, remove the lack of uniformity in the packing of the sample at the lower pressures.

The "Pendultex" method may be regarded as giving the work done during application of the compressive force, although the rapidity with which the wool recovers from compression may influence the result if the recovery is not complete before the subsequent compression commences. The calculations, as developed in the present study, are based on a large number of successive compressions, and the final result may be regarded as being influenced by those compressions, in spite of the fact that it is expressed as the work done during the first compression.

The reduction in work done during successive compressions is smaller than with the static method, since it has been found that with the static method, the final constant cycle is attained the sooner, the more slowly the compression is performed. This effect is also apparent from the fact that the number of compressions made before the pendulum recorded a constant number of swings was of the order of 150, while with the static method constancy was attained after the ninth to the fifteenth cycle.

The accuracy of the "Pendultex" method at least equals that of other methods, according to published results, but its main advantage from the point of view of routine determinations is its rapidity. Each determination requires a few minutes, and several successive determinations can be made with a considerable resulting accuracy. With the static method, on the other hand, the author's own determinations took at least three hours each for a sample.

The advantage is obvious for routine measurements in wool production studies, where large numbers have ordinarily to be dealt with. Once the instrument has been calibrated, tables may be drawn up and the resistance to compression, as defined in the present study, obtained at a glance from the number of swings recorded. The manipulation of the instrument is simple and requires little training.

In this connection it must be pointed out that with any method of determination by far the greatest portion of the time required is taken up by the preparation of the sample for testing. With the method as described, the cleansing of a series of samples for even a small-scale experiment may require several weeks. When measurements of fibre thickness and crimping are made in addition, it is obvious that rapidity is an essential requirement in the method of measuring compressibility, if large-scale determinations are to be practicable.

On the score of reproducibility of results, and the rapidity with which determinations can be repeated, the first compression has been adopted as the basis in the present study, while other workers, employing static methods, have made their observations after repeated compression. With the static

method the first compression is ill-defined and not easily reproducible, while with the dynamic method the same value may be obtained for successive determinations of the first compression of a teased sample.

In this connection it is to be noted that the value obtained by any method after constancy has been attained is dependent upon the way in which the sample has been teased out and packed into the compression compartment. It is, therefore, not sufficient to measure successive constant cycles, for reproducibility can be tested only by complete removal of the sample and re-insertion into the compression compartment. No record can be found that this fact has been borne in mind by previous workers.

(b) *The elastic behaviour of wool in bulk.*

From observations of the static method of compressing wool, the conclusion has been reached that initially the density of packing of the fibres is not uniform, but increases with successive cycles. It is therefore doubtful whether an initial cycle of compression by static methods is reproducible, and whether it has any meaning in the case of a loose mass of wool. As the pressure is raised, the packing tends to become more uniform, a fact which accounts for the observation that the volume tends to the same value at a high pressure for any cycle of compression. A part of the uniformity of packing is retained after each cycle until successive cycles are identical. Whether the final constant cycle depends on the maximum pressure to which the sample has been subjected, or upon the degree of compression, is a matter for investigation. If such is the case, the maximum pressure or degree of compression would have to be specified in each case.

Even during the final constant cycle it is clear that results obtained at low pressures should not be considered together with the later values, since on the release of the pressure, that portion of the sample nearest to the piston opens up to a greater extent than the rest of the sample. Such an effect may be expected to occur in the balloon method as well, where the friction of the fibres among themselves will oppose the release of the fibres in the centre of the mass. The result is that the wool acts like a spring at low pressures, a conclusion also reached by Schofield (1938), who stated that his sample obeyed Hooke's Law initially.

In considering the form of the curve relating pressure and volume, the compression of the sample may in the first place be regarded as consisting in the bending of individual fibres. Owing to the contacts between the fibres, the unit which bends is not primarily a whole fibre, but the element between adjacent contacts. Such elements are, however, connected to one another within a fibre, so that a certain amount of straightening, or even stretching, of the fibre may be expected. As the volume diminishes, the number of contacts and the number of elements increase, with a corresponding diminution in the length of the bending elements. The force necessary to bend an element by a certain amount is inversely proportional to the cube of its length. The two effects, viz., the increase in the number of elements and the diminution in their length will rapidly increase the resistance of the sample to compression, and hence the slope of the pressure-volume curve.

Against this a certain amount of slippage of the fibres over one another must be regarded as a possibility, and Pidgeon and van Winsen (1934) go so far as to say that "the pressure-volume relation of a mass of fibre is ultimately dependent on the ease with which they slip over one another".

Besides bending and stretching of the fibres, some torsion may be expected in view of the twist present in wool fibres. With such a complexity of factors in operation, it is doubtful whether a complete theoretical derivation of the relation between pressure and volume is possible.

Theoretical Considerations.—In spite of the complexity of the factors an attempt will be made to approach the problem with the aid of simpler cases. Two regular geometrical patterns will first be presented and simple bending only will be considered.

When an element dx of a bar is bent into an arc of radius R ,

$$\text{Bending moment} \dots\dots M = \frac{iY}{R} \dots\dots\dots (13)$$

$$\text{Energy in length } dx, \quad dE = \frac{iY}{2R^2} \cdot dx \dots\dots\dots (14)$$

where i is the "moment of inertia of cross-section", and Y is Young's modulus.

(i) *Closed Solenoid.*—Suppose that a fibre of length l and diameter d forms a *closed* solenoid of radius R . Then if v is the volume of the solenoid,

$$R = \frac{2v}{ld} \dots\dots\dots (15)$$

and from (14), since R is constant,

$$E = \frac{iYl}{2R^2} = \frac{iYl^3 \cdot d^2}{8v^2}$$

Providing the shape of the solenoid does not alter, a pressure perpendicular to the axis of the solenoid will produce a diminution in volume similar to that caused by an axial torque. Considering the wool sample to consist of a large number of such solenoids, the pressure is given by

$$p = - \frac{dE}{dv} = \frac{iYl^3 \cdot d^2}{4v^3}$$

For a fibre of circular cross-section, $i = \frac{\pi \cdot d^4}{64}$, and if m is the mass of the material and ρ the specific gravity

$$m = \frac{\pi d^2 l \rho}{4}$$

Hence

$$p = \frac{Ym^3}{4\pi^2 \rho^3 v^3} \dots\dots\dots (16)$$

Now equation (16) is identical to equation (5) except for an additive constant, which may be explained by the fact that wool fibres are bent and looped initially without stress, and in addition have in the mass a pressure among themselves in the absence of applied pressure.

It is to be noted that according to equation (16), the relation between pressure and volume depends on the total mass but not on the fibre diameter.

Although the solenoidal form is only a simple approximation to the complicated forms taken by the fibres, it is not altogether an unreasonable one. In this connection the observations of Woods (1935) are of some interest. Woods found that when single fibres were immersed in water, they relaxed and formed loops corresponding in a regular way to the original crimping. In the fibre mass a large number of such loops may be expected, and more will be formed when the mass is compressed and the fibres bend round one another, and the loops may be regarded as elementary solenoids.

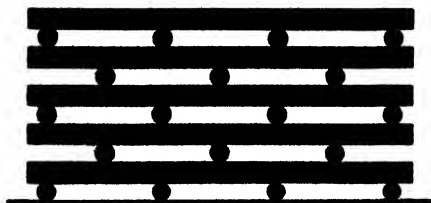
Equation (16) was derived from the condition of equation (15) viz., that the radius of curvature is proportional to the volume. The total length of fibre in a mass is of the order of 10^8 cm. per gm., and in consequence there will be a large number of fibre elements and loops. If it be assumed that for such a large number, the mean radius of curvature is proportional to the volume occupied by the mass, equation (16) giving the pressure as proportional to the inverse cube of the volume may be applied to the fibre mass (taking initial conditions into account).

When equation (15) for the solenoid is applied to a mass of fibres of 20μ diameter occupying 20 c.c. per gm., R becomes 0.04 cm. These values apply to a *closed* solenoid and are only approximate for the wool, but it is interesting to note that Woods gives the values 0.029 cm. and 0.040 cm. for the natural radius of curvature of a dry and a wet fibre of 20μ diameter respectively, values which are of the same order of magnitude as those calculated.

The above reasoning could no doubt be extended to the case of a yarn, where the fibres are twisted round one another, and their form approximates to solenoids with equally spaced turns. Provided the spacing between the turns remains constant, the radius of curvature of such a solenoid is proportional to its volume.

It is to be noted that simple bending only has been considered. In view of the twist already present in the fibres, further twisting must be considered as likely.

(ii) *Pile of rods*.—As a second geometrical pattern consider an arrangement as in the accompanying figure, where a large number of weightless rods have been piled on one another.



Let n = the number of rods,
 N = the number of layers,
 L = the length of a rod,
 d = the diameter of a rod,
 l = the total length of the rods,
 $= nL$.

The volume occupied by the pile is

$$v_0 = NL^2 \dots \dots \dots (17)$$

The distance $2b$ between adjacent contacts of two layers is

$$2b = \frac{NL}{n} \dots \dots \dots (18)$$

or from (17), $2b = \frac{v_0}{nl} \dots \dots \dots (19)$

Let a uniform pressure be applied to the top of the pile. At each point of contact the rods are bent by an amount y_1 . The new volume becomes

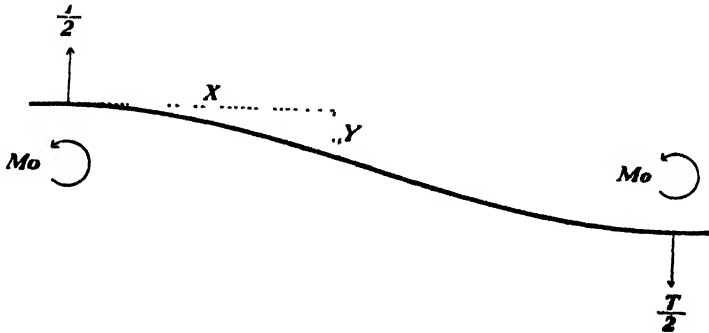
$$v = v_0 - NL^2 y_1.$$

Hence $y_1 = \frac{v_0 - v}{NL^2}$

or from (17) $y_1 = \frac{d. (v_0 - v)}{v_0} \dots \dots \dots (20)$

Let forces T act at the midpoint and ends of the portion $2b$. Then since the two halves are symmetrical, one half of length b may be considered.

At each point of contact, a force $\frac{T}{2}$ may be considered to be effective on the length b .



The bending moment at a distance x along the rod is given by $(\frac{Tx}{2} - M_0)$, where M_0 is a bending moment caused by the forces on the extension of b beyond the point of contact. But from (13), the bending moment is balanced by a moment $\frac{iY}{R}$.

Hence $\frac{iY}{R} = \frac{Tx}{2} - M_0.$

At $x = \frac{b}{2}$ there exists a point of inflexion where $\frac{1}{R} = 0$.

Hence $M_0 = \frac{Tb}{4}$, and if R is large

$$\frac{iY}{R} = iY \cdot \frac{d^2 y}{dx^2} = \frac{T}{2} \cdot (x - \frac{b}{2}) \dots \dots \dots (21)$$

$$\therefore iY \cdot \frac{dy}{dx} = \frac{T}{2} \cdot \left(\frac{x^2}{2} - \frac{bx}{2} \right), \text{ since } \frac{dy}{dx} = 0 \text{ when } x = 0,$$

$$iY \cdot \int_0^{y_1} dy = \frac{T}{2} \int_0^b \left(\frac{x^2}{2} - \frac{bx}{2} \right) dx$$

$$\text{i.e. } iYy_1 = -\frac{Tb^3}{24} \dots\dots\dots(22)$$

Now the number of contacts between two layers is equal to the square of the number of rods in a layer, and hence equals $\left(\frac{n}{N}\right)^2 = \frac{L^2}{4b^2}$, from (18).

$$\text{Hence the total force on a layer} = T \cdot \frac{L^2}{4b^2}$$

$$\begin{aligned} \text{and the pressure } p &= \frac{T \cdot L^2}{L^2 \cdot 4b^2} \\ &= \frac{T}{4b^2} \\ &= \frac{6iYy_1}{b^5}, \text{ from (22).} \end{aligned}$$

Substituting for y_1 from (20) and for b from (19),

$$p = \frac{192iYd^6l^5}{v_0^6} \cdot (v_0 - v).$$

Putting $i = \frac{\pi d^4}{64}$, and $m = \frac{\pi d^3 l \rho}{4}$, where m is the total mass, and ρ the specific gravity,

$$p = \frac{3072 \cdot Y \cdot m^5}{\pi^4 \rho^5 v_0^6} \cdot (v_0 - v) \dots\dots\dots(23)$$

The same result may be obtained by considering the energy of the system. From (14), the energy in a length dx is

$$\begin{aligned} dE &= \frac{iY}{2R^2} \cdot dx \\ &= \frac{T^2}{8iY} \cdot \left(x - \frac{b}{2}\right)^2 \cdot dx, \text{ from (21).} \end{aligned}$$

The energy in a length b is

$$\begin{aligned} E &= \frac{T^2}{8iY} \cdot \int_0^b \left(x - \frac{b}{2}\right)^2 \cdot dx \\ &= \frac{T^2 b^3}{96iY}. \end{aligned}$$

Hence the energy in a total length l is

$$\begin{aligned}
 E &= \frac{T^2 l b^2}{96iY} \\
 &= \frac{6iYly^2}{b^4}, \text{ from (22)} \\
 &= \frac{6iYld^2}{b^4 \cdot v_0^2} \cdot (v_0 - v)^2, \text{ from (20)} \\
 &= \frac{96iYl^2 d^2}{v_0^6} \cdot (v_0 - v)^2, \text{ from (19)} \\
 \text{Hence } p &= - \frac{dE}{dv} = \frac{192iYl^2 d^2}{v_0^6} \cdot (v_0 - v) \\
 &= \frac{3072Ym^5}{\pi^4 \rho^6 v_0^6} \cdot (v_0 - v) \dots \dots \dots (23)
 \end{aligned}$$

Again, as in the case of the solenoids, the relation between pressure and volume depends on the mass but not on the diameter of the rods.

For small compressions of the pile, the pressure is proportional to the reduction in volume, and it has been pointed out that the same is true for small compressions of a wool sample. It is obvious that the result obtained can be considered only in the light of small compressions, for beside the approximation made, the distance $2b$ between contacts is regarded as constant, while in practice more contacts will be formed as the fibres are bent.

From (19), the distance b is initially given by

$$b = \frac{v_0}{2ld}$$

Assuming that in the case of wool the relation holds for large degrees of compression, and defining an element length as b , the relation may be written

$$b = \frac{v}{2ld} = \frac{v}{m} \cdot \frac{\pi d \rho}{8} = 0.51 \frac{v}{m} \cdot d \dots \dots \dots (24)$$

while from (15)

$$R = \frac{2v}{ld} = \frac{v}{m} \cdot \frac{\pi d \rho}{2} = 2.04 \frac{v}{m} \cdot d$$

where m is the mass and ρ the specific gravity of wool. For a given density of packing, i.e., for a given value of $\frac{v}{m}$, the element length and the radius of curvature are proportional to the fibre diameter.

The length of a fibre element between contacts in the case of a sample of 20μ diameter occupying 20 c.c. per gm. is then 0.02 cm. This value is one-quarter of the radius of curvature obtained by analogy with a solenoid. Although an exact comparison of the two quantities is hardly valid in view of the different grounds on which the formulae have been based, the two quantities, element length and radius of curvature, may reasonably be regarded as being of the same order of magnitude. At a density of 20 c.c. per gm. the element length is about ten times the fibre diameter, and at 10 c.c. per gm. about five times the fibre diameter.

The smallest volume which the pile can occupy occurs when $2b=d$. Then

$$v_0 = ld^2 = \frac{m}{\rho} \cdot \frac{4}{\pi}$$

The smallest volume is thus $\frac{4}{\pi}$ times the volume of the material, i.e., the ratio of the area of a square to the area of the inscribed circle. Since wool fibres are arranged in all directions, the limiting volume will be even greater. This should be borne in mind when assigning a value to the constant v' of equation (5a) and equation (11), pages 123 and 124.

(iii) A less regular arrangement is required for the following treatment, in which the numerical factor has not been determined.

Suppose the wool to be compressed in a vertical direction in a container of unit area of cross-section, and let the depth at any instant be v , so that the volume occupied by the wool is v . It will be assumed that the elements of length b , formed between adjacent contacts, are the units which bend when the mass is compressed.

Let c be the vertical height occupied by an element, and consider a layer bounded by two horizontal planes a distance c apart. The layer will be one element thick, and since its area is unity, an increase in pressure dp will be equal to the increase dF in the force on each element, multiplied by the number of elements in the layer. If l is the total length of fibre, the total number of elements is $\frac{l}{b}$, and the number in the layer will be $\frac{c}{v} \cdot \frac{l}{b}$

$$\text{Hence } dp = \frac{lc}{vb} \cdot dF$$

When a bar of length b is bent by an amount dy , then

$$dF = \frac{kiY}{b^3} \cdot dy \dots \dots \dots (25)$$

where k is a numerical factor depending on the conditions of bending, i is the "moment of inertia of cross-section", and Y is Young's modulus. The relation is commonly employed in the determination of Young's modulus by the bending of a bar, and is similar to equation (22).

$$\text{Hence } dp = \frac{kiYlc}{vb^4} \cdot dy.$$

Each layer of thickness c will be reduced in thickness by an amount equal to dy , so that the reduction in volume dv is given by

$$dv = - \frac{v}{c} \cdot dy$$

$$\text{and } dy = - \frac{c}{v} \cdot dv.$$

$$\text{Hence } dp = - \frac{kiYlc^2}{v^2 \cdot b^4} \cdot dv.$$

Since the elements are arranged in all directions, the mean value of c^2 may be taken as being equal to the mean value of $\frac{b^2}{3}$ so that

$$dp = - \frac{kiYl}{3v^2b^2} \cdot dv.$$

Assuming equation (24) for the pile of rods, i.e. $b = \frac{v}{2ld}$,

$$dp = - \frac{4kiYl^3d^2}{3 \cdot v^4} \cdot dv$$

For a bar of circular cross-section, $i = \frac{\pi \cdot d^4}{64}$, hence

$$\begin{aligned} dp &= - \frac{4k\pi Yl^3d^6}{192 \cdot v^4} \cdot dv \\ &= - \frac{4kYm^3}{3\pi^2\rho^3} \cdot \frac{dv}{v^4} \end{aligned}$$

where m is the mass, and ρ the specific gravity of wool.

Integrating,

$$p = \frac{4kYm^3}{9\pi^2\rho^3} \cdot \left(\frac{1}{v^3} - \frac{1}{v_0^3} \right) \dots \dots \dots (5c)$$

and by comparison with equation (5),

$$A = \frac{4kYm^3}{9\pi^2\rho^3} \dots \dots \dots (26)$$

Now the assumptions made may be regarded as reasonable provided there are a sufficiently large number of elements. Taking 1 gm. of wool of 20μ diameter compressed into 10 c.c. per gm., the highest density employed in the present study, the mean element length may be calculated, by analogy with a pile of rods [equation (24)], to be 0.010 cm. Since the total length of fibre is 2.45×10^8 cm., the total number of elements will be 2.5×10^7 per gm. of wool, and the number in the layer of unit area of cross-section and thickness c approximately 10^4 .

Besides depending on the bending conditions, the constant k may be regarded as including a number of factors which will be briefly considered.

1. The mean element length has been taken to be equal to $\frac{v}{2ld}$ by analogy with a pile of rods. Since wool fibres are arranged in all directions, it is probable that the expression should be multiplied by some factor. For example, again consider the wool to be enclosed in a container of unit area of cross-section and depth v , and consider a spherical particle of diameter d equal to that of the fibres, moving in a vertical direction. The probability that it will strike a fibre is the area presented by the fibres projected on a horizontal plane, divided by the total area of cross-section of the container, which is unity. Since a collision will occur when the centre of a particle comes within a distance d of the axis of the fibre, the area presented by the fibres will be $2ld$, and the mean square of the projection of this area on a horizontal plane will be $\frac{2}{3} \cdot 4 \cdot l^2 \cdot d^2$. If then in travelling a distance r , the

particle strikes a fibre $\sqrt{\frac{3}{2}} \cdot 2ld$ times, then the root mean square distance between successive collisions will be given by $\sqrt{\frac{3}{2} \cdot \frac{v}{2ld}} = 0.61 \cdot \frac{v}{ld}$. If a fibre is regarded as the path of such a particle, the R.M.S. distance between successive contacts is thus $0.61 \cdot \frac{v}{ld}$. (The mean square of the projection of the area has been considered, since the substitution is made for b^2). While this calculation is only approximate, since it considers the mean value of b , it confirms the validity of the expression $\frac{v}{2ld}$ for the calculation of the element length, and shows that the resultant error takes the form of a factor which is dimensionless.

2. In the derivation a mean value for the element length b has been employed, a procedure which is valid only when b is constant. The length b will, however, follow some law of distribution, and a rigorous calculation would have to take the nature of the distribution and the extent of the variation into account. For example, in the expression $\frac{1}{b^3}$ occurring in the relation between force and deflection, it is obvious that the contribution of the shorter elements, especially those approaching d , will be large compared to the contribution of longer elements, and may reach enormous values.

3. The "moment of inertia of cross-section", i , depends upon the fibre diameter, which varies considerably within a sample, and the variation would have to be taken into account in calculating the deflection of an element. It is further to be observed that the cross-section of a wool fibre more nearly resembles an ellipse than a circle. Assuming the fibre to adjust itself so as to offer the least resistance to bending, an approximate calculation suggests that the value of i calculated by assuming circularity should be multiplied by the ratio of the minor to the major axis of the ellipse.

4. The equation

$$dF = \frac{kiY}{b^3} \cdot dy$$

assumes the deflection dy and the force dF to be perpendicular to the direction of the fibre, which will not in general be the case.

The constant k may, however, on the whole be regarded as dimensionless, and although its theoretical derivation may not be practicable, it may be obtained experimentally if the value of Y can be determined independently of the resistance to compression A . In the case of a straight fibred wool of the coarser type the measurement of Y may be practicable, e.g., by Searle's method. but in the present study no such wools were encountered.

Values of A obtained by the static method (Table 16) ranged from 6.4×10^3 to 23.4×10^3 Kg. cm.² per 5 gm. Assuming a value of, say 10 for k , Y is found to range from 2.5×10^8 dynes/cm.² to 9.2×10^8 dynes/cm.².

For Young's modulus as obtained by extension Speakman (1930) gives 3.55×10^{10} dynes/cm.² for Cotswold wool at 65 per cent. relative humidity, while the present author has found values ranging from 1.5×10^{10} dynes/cm.²

for Merino wool to 3.2×10^{10} dynes/cm.² for mohair at 70 per cent. relative humidity (van Wyk, 1932). In view of the structure of the fibre, the value of Young's modulus obtained by bending is not likely to be the same as that obtained by stretching, a point which will be considered later in connection with the effect of adsorbed water.

Assuming b to be constant and equal to $\frac{v_0}{2ld}$, the relation becomes

$$\frac{4kYm^3}{3\pi^2\rho^3v_0^2} \cdot \frac{1}{v} \left(\frac{1}{v_0} \right) \dots\dots\dots (27)$$

i.e., for compressions which are so small that no new contacts are formed, the pressure varies as the inverse first power of the volume. This result is in contrast to that obtained by the regular arrangement of rods, in the case of which the pressure was found to vary directly with the reduction in volume.

The above treatment of the problem requires that there shall be no slippage of the fibres over one another, a point to be considered later. It also assumes the density of packing to be uniform throughout the mass, explaining why the inverse cube law does not hold at small degrees of compression.

The coefficient A of equation (5) has been found to contain the mass but not the diameter of the fibres. Hence in all three cases considered, the relation between pressure and volume contained the mass but was independent of the fibre diameter.

It is to be noted that the above considerations take no account of the hysteresis loop formed between the compression and release curves. It may appear remarkable that after successive cycles the wool mass should return to its volume at zero pressure in spite of the considerable hysteresis loop. The same phenomenon occurs in the extension of single fibres, but in the case of the compression of a mass of wool the factor of friction between fibres is added to the lag of the fibres in recovering from strain. The greater part of the recovery of the mass to its original volume takes place on removal of the last traces of the pressure. During this stage the sample opens up rapidly and unevenly, and the density of packing is far from uniform. It is over the same range of low pressures that the compression curve follows Hooke's law.

It has been shown that in practice the pressure may be regarded as a linear function of the inverse cube of the volume. M. and J. Eggert (1925) appear to have found different values for the index of the volume. The author has, unfortunately, not been able to gain access to their publication, and has had to rely for his information on the quotations of other authors. It has to be emphasised, however, that the inverse cube law holds only after repeated compression by the static method, and the results at low pressures do not follow the law. The use of the quantity v_0 , suggests that these workers used the results obtained at both low and high degrees of compression. Even assuming that these factors are taken into consideration, however, the exact value 3 may not be the best-fitting one in all cases, but an additional constant is introduced when the index is deduced from the observational data. Now the conviction has already been expressed that compressibility measurements do not justify the use of an equation involving more than two unknown constants, and different values of the index may easily be found as a result of

experimental error. The other constants are at the same time affected, so that the resultant error may far exceed any that may arise from the assumption of the inverse cube law.

The Eggerts reduced the number of their unknown constants to two by taking for one constant the value of v_0 , the volume at zero applied pressure. If, as the present study has suggested, the results at low pressures do not "fit in" with the results at higher pressures, this procedure cannot be considered as entirely justifiable. In the second place, it is a matter of extreme difficulty to assess when the balloon has been suitably filled. In the present study the volume at zero pressure after repeated compression was found to be at least 30 c.c. per gm. Authors who have employed the balloon method appear to have placed samples of 4 to 5 gm. in balloons having capacities of 80 to 100 c.c. It is almost certain that the wool must have exerted some pressure on the balloon initially and the volume at zero pressure must in such cases be regarded as rather arbitrary.

In conclusion it must be observed that several interesting features of the pressure-volume relation were encountered, which required explanation. The scope of the study had, however, to be borne in mind, and only those features relevant to the study were investigated.

(c) *The arithmetical expression of compressibility.*

Since the first object in any investigation must be to express the quantity being measured in arithmetical terms, various authors have placed different interpretations on their results. M. and J. Eggert, assuming the relation

$$\phi^\gamma (\pi + \pi_0) = \pi_0 \cdot 10^\gamma$$

where π was the pressure, and ϕ ten times the ratio of the volume to the volume at zero pressure, regarded π_0 as an expression of the softness (*Weichheit*) of the wool, and γ as a measure of its pliability (*Geschmeidigkeit*).

Now wool fibres are bent and looped naturally without stress, and the "latent" pressure, π_0 , is presumably the pressure which would have been necessary to bring the fibres into this state had they been straight initially. In the fibre mass, however, the fibres are in state of strain owing to the fact that they prevent one another from taking up their normal form. Thus in the absence of applied pressure there nevertheless exists a pressure among the fibres, and this pressure will influence the determination of π_0 , the "latent" pressure of the wool. It would appear, therefore, that this "latent" pressure must partly depend on the extent to which the wool was teased out prior to compression.

Subjecting the wool to repeated cycles of compression and release will not improve the position, since it has been found with tests by both the static and dynamic methods that the pressure-volume relation after repeated compression depends as much on the extent to which the wool has been separated and on the method of insertion into the compression compartment as does the relation of the first compression. It has been suggested that owing to the difficulty of assessing when the balloon has been suitably filled, the wool initially exerts some pressure on the balloon. In such a case, the value of the "latent" pressure of the wool obtained by the balloon method must be regarded as rather arbitrary.

When $\gamma=3$, the Eggert equation becomes equation (5) of the present study, and

$$\pi_0 \cdot v_0^3 = A$$

so that

$$\frac{d\pi_0}{\pi_0} = - \frac{3 \cdot dv_0}{v_0}.$$

It is thus seen that π_0 will be very sensitive to errors in v_0 .

As regards the quantity γ as a measure of pliability, it has been found that the value may be assumed to be 3 in all cases. The effect of experimental errors on the determination of γ will be to produce a negative correlation between γ and π_0 . It is significant that the Eggerts, according to Sommer (1936), actually found a high negative correlation.

It is recommended that the value 3 should be assumed throughout, as in equation (5) or (5a), and that the constant $\frac{A}{m^3}$ be taken as a measure of the resistance to compression of the sample. Or equation (11) may be applied, taking a common value for Q which may be decided upon after the results have been obtained. In that case the coefficient P may be regarded as a measure of the elastic characteristics of the sample.

An illustration, consider the effect of the dye on the two samples of yarn tested by Larose (1934). Table 17 gives the coefficient A of equation (5a) and the coefficient P of equation (11).

TABLE 17.

The effect of the dye on the two samples of yarn tested by Larose.

Sample.	A	Ratio.	P	Ratio.	Ratio $\frac{A}{P}$
1 (undyed).....	10.71×10^6	2.13	210.0	1.91	5.10×10^4
1 (dyed).....	22.78×10^6		401.9		5.67×10^4
2 (undyed).....	3.13×10^6	2.43	60.7	2.52	5.16×10^4
2 (dyed).....	7.59×10^6		152.9		4.96×10^4
Mean.....		2.28		2.22	5.22×10^4

According to both coefficients, the effect of the dye has been practically the same for the two samples, in spite of the fact that the compressibility of one sample is three times that of the other. The table clearly illustrates the value of applying equations (5a) or (11) for finding the effect of the treatment, and for assessing the relative degrees of compressibility of the two samples.

Another illustration of the value of the equations is given by Larose's measurements on different masses of the same sample. The samples of 3.26 gm. and 4.34 gm. showed good agreement in the ratio of mass to volume at each pressure but the 2.20 gm sample showed agreement at the higher pressures only. Fitting equations (5a) and (11) to the data, the results shown in Table 18 are obtained. The constant B is equivalent to $\frac{A}{(v_0 - v)^3}$ of equation (5a).

TABLE 18.

The coefficients of equations (5a) and (11) when fitted to Larose's determinations on different masses of the same sample.

Mass.	Equation (5a).		Equation (11).		
	A m^3	B	P	Q	R
2.20 gm.....	1.98×10^5	77	138	13.1	397
3.26 gm.....	2.23×10^5	177	144	13.6	528
4.34 gm.....	2.15×10^5	168	142	13.5	521

The discrepancy in the smallest sample has reduced the coefficient A , by about 10 per cent. and the coefficient P by about 4 per cent. These differences cannot be considered serious, and can be attributed to the difficulty of handling small samples. A large discrepancy occurs, however, in the coefficients B and R of the 2.20 gm. sample, explaining why Larose found agreement only at the higher pressures. The difference points to either a systematic error in the measurement of the pressure, or to a difference in the packing into the compression compartment. Since the results given are the mean of several determinations, a systematic error in the measurement of the pressure could hardly have escaped notice, and the second of the two causes suggested may therefore be regarded as probable.

The main problem in the arithmetical expression of compressibility lies in the basis on which different wools have to be compared. Thus, samples may be compared on the basis of equal masses, of equal lengths of fibre, or of equal values of r_0 , the volume at zero applied pressure. Now the coefficient A of equation (5) includes the cube of the mass, and the methods of equal masses and of equal lengths of fibre will probably yield coefficients differing in the sixth power of the fibre diameter, so that the fibre diameter can be eliminated and the two coefficients reduced to a common value.

Had the volume r_0 at zero applied pressure been well-defined and reproducible, this would undoubtedly have been the best basis for comparing different wools, at least theoretically. It is the basis adopted by the Eggerts (1925), and it will be shown to be the most suitable for evaluating the work done during compression. Where the density of packing of the fibres has already been increased, as in the case of felt, cloth and even yarn, such a method is practicable, but the lack of reproducible initial conditions in the case of a loose mass, and the difference between the pressure-volume relation at low and high degrees of compression, as has been stressed, precludes the accurate comparison of wools on the basis of equal values of r_0 . On such a basis the most satisfactory method would probably be to calculate r_0 from the relation fitted to the later values.

With the dynamic method employed in the present study, samples can be compared only on the basis of equal masses, and this basis has been adopted in the present study. When the compressibility of a sample is estimated by hand, it is probable that the judge will grasp a constant volume, i.e., a constant value of r_0 . On the other hand, the manufacturer employs

the weight as a fundamental quantity. Which of the two methods of comparison conforms to manufacturing practice remains a matter for investigation.

The method of expressing compressibility by means of the work done during compression.—Some previous investigators have attempted to express the compressional characteristics of a sample by means of the work done during compression, and have compared different samples by the work done in compressing the samples between the same limits of pressure. Since the work done is an integration of the function $p.dv$ between volume limits, the question arises as to whether the limits employed are justifiable.

When different masses of the *same* sample are to be compared it is clearly necessary that the limits should be so chosen that the work done is proportional to the mass. The work done per unit mass will then be the same whatever the mass chosen for the determination. Since the ratio of mass to volume is the same for all masses of the same sample at any pressure, and therefore at zero pressure, the above condition will be fulfilled when the limits chosen are v_0 , the volume at zero pressure, and some fraction of v_0 which is the same for all masses. For the same sample, such volume limits will also be given by equal pressure limits.

When two different samples are to be compared on the basis of equal masses, the ratio of mass to volume is not the same for the two samples, and the theoretical and practical significance of a comparison of the work done between volume limits given by equal pressure limits is not quite clear. When the lower limits are v_0 and the upper limits the same fraction of v_0 for the two samples, the ratio of the work done per unit mass is the ratio of the coefficients of resistance to compression [e.g., A in equation (5)], multiplied by a factor involving the respective values of v_0 . Thus on the basis of equation (5), the ratio of the work done per unit mass is given by

$$\frac{W_1}{W_2} = \frac{A_1}{A_2} \cdot \left\{ \frac{v_0(2)}{v_0(1)} \right\}^2 \dots\dots\dots (28)$$

As a practical illustration, the data of Larose may again be utilised. When equations are fitted to the data, the work done may be evaluated between any limits of volume. The results are given in Table 19.

TABLE 19.

The work done, in arbitrary units, in compressing four samples of yarn at 50 per cent. relative humidity, as calculated for various limits of volume from data by Larose.

Sample.	VOLUME LIMITS GIVEN BY—			
	Equal Limits of Pressure.		Equal Limits of $\frac{v}{v_0}$	
	W	$\frac{W}{(v_0-v)}$	W	Ratio Dyed/Undyed.
1 (undyed).....	4,016	109	4,658	1.67
1 (dyed).....	4,196	99	7,770	
2 (undyed).....	3,288	112	2,282	1.62
2 (dyed).....	3,780	108	3,700	

Limits of volume given by the same limits of pressure give the work done in the same order as the coefficients of resistance to compression, but not in the same relative magnitude (Table 17). The ratio $\frac{W}{v_0}$ or $\frac{W}{v_0 - v}$ is the equivalent result obtained from the area of the curve when pressure is plotted as a function of $\frac{v}{v_0}$, a method employed by Winson. This procedure is clearly not sufficient for comparing the samples, when equal pressure limits are employed, as the values are practically the same.

When the integration is performed between the same limits of $\frac{v}{v_0}$ the ratio of the dyed to the undyed values is the same for the two samples, although somewhat smaller than the ratios given by coefficients A and P on account of the smaller volume occupied by the undyed sample at zero pressure in each case.

When the samples are compared on the basis of the same limits of volume, the ratio of the work done, assuming the samples to follow equation (5), is equal to the ratio of the coefficients A , multiplied by a factor which contains the cubes of the volume at zero pressure. This is the method employed in the "Pendultex" apparatus, but it is not satisfactory in the case of static methods, since the volume occupied by one sample at the highest pressure employed may still be greater than the volume occupied by another sample of equal mass at zero pressure.

When it is desired to compare samples by the work done in compressing them by static methods, the most profitable limits of volume appear to be those given by the same limits of $\frac{v}{v_0}$. It is recommended that the calculated value of v_0 should be employed for this purpose.

The method of expressing resilience.—Larose considered that the compression curve alone was sufficient to specify the degree of harshness of his samples. The coefficient P , for example, as given in Table 17, had the values 60.7, 152.9, 210.0 and 401.9 when the samples were placed in increasing order of harshness as tactually estimated.

Henning regarded the compression curve as of supreme importance, arguing on the basis that the wool having a superior compression curve would also have a superior release curve. This argument may be valid in the case of untreated wools, but the possibility exists that the extent of the hysteresis may be altered by chemical treatment. In this connection attention must be drawn to the work of Speakman, Stott and Chang (1933) on the load-extension curves of wool fibres immersed in acids and alkalis and in water at various temperatures.

While the compression curve may readily be represented by an analytical equation, this is hardly practicable in the case of the release curve, and the only convenient method of studying the release conditions and the hysteresis effect is by means of the work done. Thus Winson took the area of the hysteresis loop as a measure of resilience, stating that this quantity appeared to correspond to the trade impression of "springiness". As other authors have pointed out, the use of the term "resilience" cannot be justified in this sense, nor can its use by Schiefer (1933) as the difference in the work done between the compression and release operations expressed as a ratio of the

work done during compression. Resilience is usually defined as the energy stored in a strained elastic body. Granting the importance of the hysteresis, attention must, however, again be drawn to the questionable practice of evaluating the work done between volume limits given by equal limits of pressure.

In this connection consider the work done during compression and release in the case of four yarns tested by Larose, as given in Table 20.

TABLE 20.

The work done, in arbitrary units, in compressing and releasing the four samples of yarn tested by Larose at 50 per cent. relative humidity.

sample.	WORK DONE.			
	Compression.	Release.	Difference.	Percentage Difference.
				Per Cent.
1 (undyed).....	4,016	1,872	2,144	53
1 (dyed).....	4,196	1,956	2,240	53
2 (undyed).....	3,288	1,744	1,544	47
2 (dyed).....	3,780	1,612	2,168	57

The work done as given in Table 20 was evaluated between volume limits given by equal pressure limits, the form in which the data were given. The values for difference and percentage difference are too close together to be of practical value, and the results suggest that no difference exists between the samples as regards percentage hysteresis. According to Winson, three of the samples would have to be classed as having the same springiness. It has been demonstrated that the most profitable method of evaluating the work done during compression is by means of volume limits given by the same limits of $\frac{v}{v_0}$. It is reasonable to expect that the same applies to the hysteresis.

Such a method corresponds to that successfully employed by Speakman, Stott and Chang (1933) in their work on the extension of single fibres, since each fibre was extended by 30 per cent. of its original length.

The value of the compression curve has been demonstrated, and applications will be found in Part II of this paper, but the practical significance of the hysteresis effect needs investigation. Such determinations as have been made can be regarded as of little value owing to the failure of the investigators concerned to employ the correct limits of volume in evaluating the work done.

APPENDIX A.

Method employed for fitting the equation

$$p = Pe^{\frac{Qm}{(v-\bar{v})}} - R \dots \dots \dots (11)$$

Fitting was accomplished in two stages. First approximate values of the constants P , Q and R were derived, and these were next improved upon by successive approximation.

1. *Approximate values.*

Pressure was plotted as a function of $\frac{m}{(v-v')}$, and a smooth free-hand curve was drawn through the points. From the curve, pressure was read off at equal intervals of $\frac{m}{(v-v')}$, and designated $p_0, p_1, p_2 \dots$. For the observations to fit the equation a linear relation had to exist between p_n and p_{n+1} , a relation expressible in the form

$$p_n + R = e^x \cdot (p_{n+1} + R),$$

where x was the interval between successive values of $\frac{m}{(v-v')}$. The constants Q and R could thus be determined, and P could be calculated from the original observations.

2. The constants so obtained were regarded as approximations P', Q' and R' such that

$$P = P' + \alpha$$

$$Q = Q' + \beta$$

$$R = R' + \gamma$$

Now if $p = f(P, Q, R, \frac{m}{(v-v')})$ where p was the observed value and p' was the value of p given by the approximate constants, the values of α, β and γ could be obtained by making $\Sigma (\alpha \cdot \frac{df}{dP} + \beta \cdot \frac{df}{dQ} + \gamma \cdot \frac{df}{dR} + p' - p)^2$ a minimum. (See Scarborough, 1930).

It was found necessary to repeat the calculation at least three times before sufficiently small values for the corrections α, β and γ were obtained.

With the static cylinder and piston method employed in the present study, the pressures were given by weights and were therefore determined with negligible error. The equation was consequently fitted by regarding p as the independent variable, and taking

$$\frac{m}{(v-v')} = f(P, Q, R, p).$$

For the satisfactory fitting of any equation it is necessary that one of the variables should have been measured with negligible error. This condition was not fulfilled by the results published by some previous investigators, since certain measurements entered into the evaluation of both pressure and volume. Both variables must therefore be considered as having been measured with sensibly the same error.

APPENDIX B.

Table of the indefinite integral $\int_0^1 e^x \cdot dx$, for values of x from 0.4 to 2.5 at intervals of 0.001, employed for evaluating the work done in compressing a wool sample, when the relation between pressure and volume is given by

$$p = P e^{\frac{Qm}{(v-v')}} - R \dots \dots \dots (11)$$

THE COMPRESSIBILITY OF WOOL.

π	0	.001	.002	.003	.004	.005	.006	.007	.008	.009
0.40	2.6236	2.6114	2.5993	2.5873	2.5754	2.5636	2.5518	2.5401	2.5285	2.5169
0.41	2.5054	2.4940	2.4826	2.4713	2.4601	2.4489	2.4378	2.4268	2.4158	2.4049
0.42	2.3941	2.3833	2.3726	2.3619	2.3513	2.3407	2.3303	2.3198	2.3095	2.2991
0.43	2.2880	2.2787	2.2685	2.2584	2.2484	2.2384	2.2285	2.2186	2.2087	2.1980
0.44	2.1892	2.1795	2.1699	2.1603	2.1508	2.1413	2.1319	2.1225	2.1131	2.1039
0.45	2.0946	2.0854	2.0762	2.0671	2.0580	2.0490	2.0400	2.0311	2.0222	2.0133
0.46	2.0045	1.9958	1.9870	1.9783	1.9697	1.9611	1.9525	1.9440	1.9355	1.9270
0.47	1.9186	1.9102	1.9019	1.8936	1.8853	1.8771	1.8689	1.8608	1.8526	1.8445
0.48	1.8365	1.8285	1.8205	1.8126	1.8047	1.7968	1.7889	1.7811	1.7733	1.7656
0.49	1.7579	1.7502	1.7426	1.7350	1.7274	1.7198	1.7123	1.7048	1.6974	1.6899
0.50	1.6825	1.6751	1.6678	1.6605	1.6532	1.6459	1.6387	1.6315	1.6243	1.6171
0.51	1.6100	1.6029	1.5959	1.5888	1.5818	1.5748	1.5679	1.5609	1.5540	1.5472
0.52	1.5403	1.5335	1.5267	1.5199	1.5131	1.5064	1.4997	1.4930	1.4864	1.4797
0.53	1.4731	1.4665	1.4600	1.4534	1.4469	1.4404	1.4339	1.4275	1.4211	1.4147
0.54	1.4083	1.4019	1.3956	1.3893	1.3830	1.3767	1.3704	1.3642	1.3580	1.3518
0.55	1.3457	1.3395	1.3334	1.3273	1.3212	1.3151	1.3090	1.3030	1.2970	1.2910
0.56	1.2850	1.2791	1.2732	1.2672	1.2613	1.2555	1.2496	1.2437	1.2379	1.2321
0.57	1.2263	1.2206	1.2148	1.2091	1.2034	1.1976	1.1920	1.1863	1.1806	1.1750
0.58	1.1694	1.1638	1.1582	1.1527	1.1471	1.1416	1.1361	1.1306	1.1251	1.1196
0.59	1.1141	1.1087	1.1033	1.0979	1.0925	1.0871	1.0817	1.0764	1.0711	1.0657
0.60	1.0694	1.0652	1.0499	1.0446	1.0394	1.0342	1.0289	1.0237	1.0185	1.0134
0.61	1.0082	1.0031	0.9979	0.9928	0.9877	0.9826	0.9776	0.9725	0.9674	0.9624
0.62	0.9574	0.9524	0.9474	0.9424	0.9374	0.9324	0.9275	0.9226	0.9177	0.9128
0.63	0.9079	0.9030	0.8981	0.8932	0.8884	0.8835	0.8787	0.8739	0.8691	0.8643
0.64	0.8595	0.8548	0.8500	0.8453	0.8406	0.8358	0.8311	0.8264	0.8217	0.8171
0.65	0.8124	0.8078	0.8031	0.7985	0.7939	0.7893	0.7847	0.7801	0.7755	0.7709
0.66	0.7618	0.7573	0.7528	0.7483	0.7438	0.7393	0.7348	0.7303	0.7258	0.7213
0.67	0.7214	0.7169	0.7125	0.7081	0.7037	0.6993	0.6949	0.6905	0.6861	0.6817
0.68	0.6774	0.6730	0.6687	0.6644	0.6601	0.6558	0.6515	0.6472	0.6429	0.6386
0.69	0.6343	0.6301	0.6258	0.6216	0.6174	0.6132	0.6090	0.6047	0.6005	0.5964
0.70	0.5922	0.5880	0.5839	0.5797	0.5756	0.5714	0.5673	0.5632	0.5591	0.5550
0.71	0.5509	0.5468	0.5427	0.5386	0.5346	0.5305	0.5265	0.5224	0.5184	0.5144
0.72	0.5104	0.5064	0.5024	0.4984	0.4944	0.4904	0.4864	0.4825	0.4785	0.4746
0.73	0.4707	0.4667	0.4628	0.4589	0.4550	0.4511	0.4472	0.4433	0.4394	0.4355
0.74	0.4317	0.4278	0.4240	0.4201	0.4163	0.4124	0.4086	0.4048	0.4010	0.3972
0.75	0.3934	0.3896	0.3858	0.3820	0.3783	0.3745	0.3707	0.3670	0.3632	0.3595
0.76	0.3558	0.3521	0.3483	0.3446	0.3409	0.3372	0.3335	0.3298	0.3262	0.3225
0.77	0.3188	0.3152	0.3115	0.3079	0.3042	0.3006	0.2969	0.2933	0.2897	0.2861
0.78	0.2825	0.2789	0.2753	0.2717	0.2681	0.2645	0.2610	0.2574	0.2538	0.2503
0.79	0.2467	0.2432	0.2397	0.2361	0.2326	0.2291	0.2256	0.2220	0.2185	0.2150
0.80	0.2116	0.2081	0.2046	0.2011	0.1976	0.1942	0.1907	0.1873	0.1838	0.1804
0.81	0.1769	0.1735	0.1701	0.1666	0.1632	0.1598	0.1564	0.1530	0.1496	0.1462
0.83	0.1428	0.1394	0.1361	0.1327	0.1293	0.1259	0.1226	0.1192	0.1159	0.1125

z	0	-001	-002	-003	-004	-005	-006	-007	-008	-009
0.83	0.1092	0.1059	0.1025	0.0992	0.0959	0.0926	0.0893	0.0860	0.0827	0.0794
0.84	0.0761	0.0728	0.0695	0.0662	0.0630	0.0597	0.0564	0.0532	0.0499	0.0467
0.85	0.0434	0.0402	0.0369	0.0337	0.0305	0.0273	0.0240	0.0208	0.0176	0.0144
0.86	0.0112	0.0080	0.0048	0.0016	0.0015	0.0047	0.0079	0.0111	0.0142	0.0174
0.87	0.0206	0.0237	0.0269	0.0300	0.0331	0.0363	0.0394	0.0425	0.0457	0.0488
0.88	0.0519	0.0500	0.0581	0.0612	0.0643	0.0674	0.0705	0.0736	0.0767	0.0798
0.89	0.0829	0.0859	0.0890	0.0921	0.0951	0.0982	0.1013	0.1043	0.1074	0.1104
0.90	0.1134	0.1165	0.1195	0.1225	0.1256	0.1286	0.1316	0.1346	0.1376	0.1406
0.91	0.1436	0.1466	0.1496	0.1526	0.1556	0.1586	0.1616	0.1645	0.1675	0.1705
0.92	0.1735	0.1764	0.1794	0.1823	0.1853	0.1882	0.1912	0.1941	0.1971	0.2000
0.93	0.2029	0.2059	0.2088	0.2117	0.2146	0.2175	0.2205	0.2234	0.2263	0.2292
0.94	0.2321	0.2350	0.2379	0.2408	0.2436	0.2465	0.2494	0.2523	0.2552	0.2580
0.95	0.2609	0.2638	0.2666	0.2695	0.2723	0.2752	0.2780	0.2809	0.2837	0.2866
0.96	0.2894	0.2922	0.2950	0.2979	0.3007	0.3035	0.3063	0.3091	0.3120	0.3148
0.97	0.3176	0.3204	0.3232	0.3260	0.3288	0.3315	0.3343	0.3371	0.3399	0.3427
0.98	0.3455	0.3482	0.3510	0.3538	0.3565	0.3593	0.3621	0.3648	0.3676	0.3703
0.99	0.3731	0.3758	0.3785	0.3813	0.3840	0.3868	0.3895	0.3922	0.3949	0.3977
1.00	0.4004	0.4031	0.4058	0.4085	0.4112	0.4139	0.4166	0.4193	0.4220	0.4247
1.01	0.4274	0.4301	0.4328	0.4355	0.4382	0.4409	0.4435	0.4462	0.4489	0.4515
1.02	0.4542	0.4569	0.4595	0.4622	0.4649	0.4675	0.4702	0.4728	0.4755	0.4781
1.03	0.4807	0.4834	0.4860	0.4887	0.4913	0.4939	0.4965	0.4992	0.5018	0.5044
1.04	0.5070	0.5096	0.5122	0.5149	0.5175	0.5201	0.5227	0.5253	0.5279	0.5305
1.05	0.5331	0.5356	0.5382	0.5408	0.5434	0.5460	0.5486	0.5511	0.5537	0.5563
1.06	0.5589	0.5614	0.5640	0.5666	0.5691	0.5717	0.5742	0.5768	0.5793	0.5819
1.07	0.5844	0.5870	0.5895	0.5921	0.5946	0.5971	0.5997	0.6022	0.6047	0.6073
1.08	0.6098	0.6123	0.6148	0.6173	0.6199	0.6224	0.6249	0.6274	0.6299	0.6324
1.09	0.6349	0.6374	0.6399	0.6424	0.6449	0.6474	0.6499	0.6524	0.6549	0.6574
1.10	0.6598	0.6623	0.6648	0.6673	0.6697	0.6722	0.6747	0.6771	0.6796	0.6821
1.11	0.6846	0.6870	0.6895	0.6919	0.6944	0.6968	0.6993	0.7017	0.7042	0.7066
1.12	0.7091	0.7115	0.7139	0.7164	0.7188	0.7212	0.7237	0.7261	0.7285	0.7310
1.13	0.7334	0.7358	0.7382	0.7406	0.7431	0.7455	0.7479	0.7503	0.7527	0.7551
1.14	0.7575	0.7599	0.7623	0.7647	0.7671	0.7695	0.7719	0.7743	0.7767	0.7791
1.15	0.7815	0.7839	0.7862	0.7886	0.7910	0.7934	0.7958	0.7981	0.8005	0.8029
1.16	0.8053	0.8076	0.8100	0.8123	0.8147	0.8171	0.8194	0.8218	0.8241	0.8265
1.17	0.8289	0.8312	0.8335	0.8359	0.8382	0.8406	0.8429	0.8452	0.8476	0.8499
1.18	0.8523	0.8546	0.8569	0.8593	0.8616	0.8639	0.8662	0.8686	0.8709	0.8732
1.19	0.8755	0.8778	0.8801	0.8825	0.8848	0.8871	0.8894	0.8917	0.8940	0.8963
1.20	0.8986	0.9009	0.9032	0.9055	0.9078	0.9101	0.9124	0.9147	0.9170	0.9193
1.21	0.9215	0.9238	0.9261	0.9284	0.9307	0.9329	0.9352	0.9375	0.9398	0.9420
1.22	0.9443	0.9466	0.9488	0.9511	0.9534	0.9556	0.9579	0.9602	0.9624	0.9647
1.23	0.9669	0.9692	0.9714	0.9737	0.9759	0.9782	0.9804	0.9827	0.9849	0.9872
1.24	0.9894	0.9917	0.9939	0.9961	0.9983	1.0006	1.0028	1.0050	1.0073	1.0095
1.25	1.0117	1.0140	1.0162	1.0184	1.0206	1.0228	1.0251	1.0273	1.0295	1.0317

x	0	.001	.002	.003	.004	.005	.006	.007	.008	.009
1.26	1.0339	1.0361	1.0383	1.0405	1.0427	1.0450	1.0472	1.0494	1.0516	1.0538
1.27	1.0560	1.0582	1.0604	1.0625	1.0647	1.0669	1.0691	1.0713	1.0735	1.0757
1.28	1.0779	1.0801	1.0822	1.0844	1.0866	1.0888	1.0910	1.0931	1.0953	1.0975
1.29	1.0997	1.1018	1.1040	1.1062	1.1083	1.1105	1.1126	1.1148	1.1170	1.1191
1.30	1.1213	1.1235	1.1256	1.1278	1.1299	1.1321	1.1342	1.1364	1.1385	1.1407
1.31	1.1428	1.1450	1.1471	1.1492	1.1514	1.1535	1.1557	1.1578	1.1599	1.1621
1.32	1.1642	1.1663	1.1685	1.1706	1.1727	1.1749	1.1770	1.1791	1.1812	1.1834
1.33	1.1855	1.1876	1.1897	1.1918	1.1939	1.1961	1.1982	1.2003	1.2024	1.2045
1.34	1.2066	1.2087	1.2108	1.2129	1.2151	1.2172	1.2193	1.2214	1.2235	1.2256
1.35	1.2277	1.2298	1.2319	1.2339	1.2360	1.2381	1.2402	1.2423	1.2444	1.2465
1.36	1.2486	1.2507	1.2527	1.2548	1.2569	1.2590	1.2611	1.2632	1.2652	1.2673
1.37	1.2693	1.2715	1.2735	1.2756	1.2777	1.2797	1.2818	1.2839	1.2859	1.2880
1.38	1.2901	1.2921	1.2942	1.2963	1.2983	1.3004	1.3024	1.3045	1.3066	1.3086
1.39	1.3107	1.3127	1.3148	1.3168	1.3189	1.3209	1.3230	1.3250	1.3271	1.3291
1.40	1.3312	1.3332	1.3352	1.3373	1.3393	1.3413	1.3434	1.3454	1.3475	1.3495
1.41	1.3515	1.3536	1.3556	1.3576	1.3596	1.3617	1.3637	1.3657	1.3677	1.3698
1.42	1.3718	1.3738	1.3758	1.3778	1.3799	1.3819	1.3839	1.3859	1.3879	1.3900
1.43	1.3920	1.3940	1.3960	1.3980	1.4000	1.4020	1.4040	1.4060	1.4080	1.4100
1.44	1.4120	1.4140	1.4160	1.4180	1.4200	1.4220	1.4240	1.4260	1.4280	1.4300
1.45	1.4320	1.4340	1.4360	1.4380	1.4400	1.4420	1.4440	1.4459	1.4479	1.4499
1.46	1.4519	1.4539	1.4559	1.4578	1.4598	1.4618	1.4638	1.4658	1.4677	1.4697
1.47	1.4717	1.4737	1.4756	1.4776	1.4796	1.4815	1.4835	1.4855	1.4875	1.4894
1.48	1.4914	1.4934	1.4953	1.4973	1.4992	1.5012	1.5032	1.5051	1.5071	1.5090
1.49	1.5110	1.5130	1.5149	1.5169	1.5188	1.5208	1.5227	1.5247	1.5266	1.5286
1.50	1.5305	1.5325	1.5344	1.5364	1.5383	1.5402	1.5422	1.5441	1.5461	1.5480
1.51	1.5500	1.5519	1.5538	1.5558	1.5577	1.5596	1.5616	1.5635	1.5654	1.5674
1.52	1.5693	1.5712	1.5732	1.5751	1.5770	1.5789	1.5809	1.5828	1.5847	1.5867
1.53	1.5886	1.5905	1.5924	1.5943	1.5963	1.5982	1.6001	1.6020	1.6039	1.6058
1.54	1.6078	1.6097	1.6116	1.6135	1.6154	1.6173	1.6192	1.6211	1.6230	1.6250
1.55	1.6269	1.6288	1.6307	1.6326	1.6345	1.6364	1.6383	1.6402	1.6421	1.6440
1.56	1.6459	1.6478	1.6497	1.6516	1.6535	1.6554	1.6573	1.6591	1.6610	1.6629
1.57	1.6648	1.6667	1.6686	1.6705	1.6724	1.6743	1.6762	1.6780	1.6799	1.6818
1.58	1.6856	1.6875	1.6893	1.6912	1.6931	1.6950	1.6969	1.6987	1.7006	1.7025
1.59	1.7044	1.7062	1.7081	1.7100	1.7119	1.7137	1.7156	1.7175	1.7193	1.7212
1.60	1.7231	1.7249	1.7268	1.7287	1.7305	1.7324	1.7343	1.7361	1.7380	1.7398
1.61	1.7417	1.7436	1.7454	1.7473	1.7491	1.7510	1.7529	1.7547	1.7566	1.7584
1.62	1.7584	1.7621	1.7640	1.7658	1.7677	1.7695	1.7714	1.7732	1.7751	1.7769
1.63	1.7769	1.7788	1.7806	1.7825	1.7843	1.7862	1.7880	1.7917	1.7935	1.7954
1.64	1.7954	1.7972	1.7990	1.8009	1.8027	1.8046	1.8064	1.8082	1.8101	1.8119
1.65	1.8137	1.8156	1.8174	1.8192	1.8211	1.8229	1.8247	1.8265	1.8284	1.8302
1.66	1.8320	1.8339	1.8357	1.8375	1.8393	1.8412	1.8430	1.8448	1.8466	1.8484
1.67	1.8503	1.8521	1.8539	1.8557	1.8575	1.8594	1.8612	1.8630	1.8648	1.8666
1.68	1.8684	1.8702	1.8721	1.8739	1.8757	1.8775	1.8793	1.8810	1.8829	1.8847

x	0	·001	·002	·003	·004	·005	·006	·007	·008	·009
1·69	1·8865	1·8883	1·8901	1·8920	1·8938	1·8956	1·8974	1·8992	1·9010	1·9028
1·70	1·8940	1·8964	1·8982	1·9000	1·9018	1·9036	1·9054	1·9072	1·9090	1·9208
1·71	1·9226	1·9243	1·9261	1·9279	1·9297	1·9315	1·9333	1·9351	1·9369	1·9387
1·72	1·9405	1·9423	1·9440	1·9458	1·9476	1·9494	1·9512	1·9530	1·9548	1·9565
1·73	1·9583	1·9601	1·9619	1·9637	1·9654	1·9672	1·9690	1·9708	1·9726	1·9743
1·74	1·9761	1·9779	1·9797	1·9814	1·9832	1·9850	1·9868	1·9785	1·9903	1·9921
1·75	1·9939	1·9956	1·9974	1·9992	2·0010	2·0027	2·0045	2·0062	2·0080	2·0098
1·76	2·0115	2·0133	2·0151	2·0168	2·0186	2·0203	2·0221	2·0239	2·0256	2·0274
1·77	2·0292	2·0309	2·0327	2·0344	2·0362	2·0379	2·0397	2·0415	2·0432	2·0450
1·78	2·0467	2·0485	2·0502	2·0520	2·0538	2·0555	2·0573	2·0590	2·0608	2·0626
1·79	2·0642	2·0660	2·0677	2·0695	2·0712	2·0730	2·0747	2·0765	2·0782	2·0799
1·80	2·0817	2·0834	2·0852	2·0869	2·0887	2·0904	2·0921	2·0939	2·0956	2·0974
1·81	2·0991	2·1008	2·1026	2·1043	2·1060	2·1078	2·1095	2·1112	2·1130	2·1147
1·82	2·1164	2·1182	2·1199	2·1216	2·1234	2·1251	2·1268	2·1286	2·1303	2·1320
1·83	2·1337	2·1355	2·1372	2·1389	2·1406	2·1424	2·1441	2·1458	2·1475	2·1493
1·84	2·1510	2·1527	2·1544	2·1561	2·1579	2·1596	2·1613	2·1630	2·1647	2·1665
1·85	2·1682	2·1699	2·1716	2·1733	2·1750	2·1768	2·1785	2·1802	2·1819	2·1836
1·86	2·1853	2·1870	2·1887	2·1905	2·1922	2·1939	2·1956	2·1973	2·1990	2·2007
1·87	2·2024	2·2041	2·2058	2·2075	2·2092	2·2109	2·2126	2·2144	2·2161	2·2178
1·88	2·2195	2·2212	2·2229	2·2246	2·2263	2·2280	2·2297	2·2314	2·2331	2·2348
1·89	2·2365	2·2382	2·2399	2·2416	2·2432	2·2449	2·2466	2·2483	2·2500	2·2517
1·90	2·2534	2·2551	2·2568	2·2585	2·2602	2·2619	2·2636	2·2652	2·2669	2·2686
1·91	2·2703	2·2720	2·2737	2·2754	2·2771	2·2787	2·2804	2·2821	2·2838	2·2855
1·92	2·2872	2·2889	2·2905	2·2922	2·2939	2·2956	2·2973	2·2989	2·3006	2·3023
1·93	2·3040	2·3057	2·3073	2·3090	2·3107	2·3124	2·3140	2·3157	2·3174	2·3191
1·94	2·3208	2·3224	2·3241	2·3258	2·3274	2·3291	2·3308	2·3325	2·3341	2·3358
1·95	2·3375	2·3391	2·3408	2·3425	2·3441	2·3458	2·3475	2·3492	2·3508	2·3525
1·96	2·3542	2·3558	2·3575	2·3591	2·3608	2·3625	2·3641	2·3658	2·3675	2·3691
1·97	2·3708	2·3724	2·3741	2·3758	2·3774	2·3791	2·3807	2·3824	2·3841	2·3857
1·98	2·3874	2·3890	2·3907	2·3923	2·3940	2·3957	2·3973	2·3990	2·4006	2·4023
1·99	2·4039	2·4056	2·4072	2·4089	2·4105	2·4122	2·4138	2·4155	2·4171	2·4188
2·00	2·4204	2·4221	2·4237	2·4254	2·4270	2·4287	2·4303	2·4320	2·4336	2·4353
2·01	2·4369	2·4385	2·4402	2·4418	2·4435	2·4451	2·4468	2·4484	2·4500	2·4517
2·02	2·4533	2·4550	2·4566	2·4582	2·4599	2·4615	2·4632	2·4648	2·4664	2·4681
2·03	2·4697	2·4714	2·4730	2·4746	2·4763	2·4779	2·4795	2·4812	2·4828	2·4844
2·04	2·4861	2·4877	2·4893	2·4910	2·4926	2·4942	2·4958	2·4975	2·4991	2·5007
2·05	2·5024	2·5040	2·5056	2·5073	2·5089	2·5105	2·5121	2·5138	2·5154	2·5170
2·06	2·5186	2·5203	2·5219	2·5235	2·5251	2·5268	2·5284	2·5300	2·5316	2·5332
2·07	2·5349	2·5365	2·5381	2·5397	2·5413	2·5430	2·5446	2·5462	2·5478	2·5494
2·08	2·5511	2·5527	2·5543	2·5559	2·5575	2·5591	2·5608	2·5624	2·5640	2·5656
2·09	2·5672	2·5688	2·5704	2·5721	2·5737	2·5753	2·5769	2·5785	2·5801	2·5817
2·10	2·5833	2·5849	2·5865	2·5882	2·5898	2·5914	2·5930	2·5946	2·5962	2·5978
2·11	2·5994	2·6010	2·6026	2·6042	2·6058	2·6074	2·6090	2·6106	2·6122	2·6139

THE COMPRESSIBILITY OF WOOL.

π	0	.001	.002	.003	.004	.005	.006	.007	.008	.009
2.12	2.6155	2.6171	2.6187	2.6203	2.6219	2.6235	2.6251	2.6267	2.6283	2.6299
2.13	2.6315	2.6331	2.6347	2.6363	2.6379	2.6395	2.6411	2.6426	2.6442	2.6458
2.14	2.6474	2.6490	2.6506	2.6522	2.6538	2.6554	2.6570	2.6586	2.6602	2.6618
2.15	2.6634	2.6650	2.6666	2.6682	2.6697	2.6713	2.6729	2.6745	2.6761	2.6777
2.16	2.6793	2.6809	2.6825	2.6840	2.6856	2.6872	2.6888	2.6904	2.6920	2.6936
2.17	2.6952	2.6967	2.6983	2.6999	2.7015	2.7031	2.7047	2.7062	2.7078	2.7094
2.18	2.7110	2.7126	2.7142	2.7157	2.7173	2.7189	2.7205	2.7221	2.7236	2.7252
2.19	2.7268	2.7284	2.7300	2.7315	2.7331	2.7347	2.7363	2.7378	2.7394	2.7410
2.20	2.7426	2.7441	2.7457	2.7473	2.7489	2.7504	2.7520	2.7536	2.7552	2.7567
2.21	2.7583	2.7599	2.7614	2.7630	2.7646	2.7662	2.7677	2.7693	2.7709	2.7724
2.22	2.7740	2.7756	2.7771	2.7787	2.7803	2.7818	2.7834	2.7850	2.7866	2.7881
2.23	2.7897	2.7913	2.7928	2.7944	2.7959	2.7975	2.7991	2.8006	2.8022	2.8038
2.24	2.8053	2.8069	2.8085	2.8100	2.8116	2.8131	2.8147	2.8163	2.8178	2.8194
2.25	2.8209	2.8225	2.8241	2.8256	2.8272	2.8287	2.8303	2.8318	2.8334	2.8350
2.26	2.8365	2.8381	2.8396	2.8412	2.8427	2.8443	2.8459	2.8474	2.8490	2.8505
2.27	2.8521	2.8536	2.8552	2.8567	2.8583	2.8598	2.8614	2.8629	2.8645	2.8660
2.28	2.8676	2.8691	2.8707	2.8722	2.8738	2.8753	2.8769	2.8784	2.8800	2.8815
2.29	2.8831	2.8846	2.8862	2.8877	2.8893	2.8908	2.8924	2.8939	2.8955	2.8970
2.30	2.8985	2.9001	2.9016	2.9032	2.9047	2.9063	2.9078	2.9093	2.9109	2.9124
2.31	2.9140	2.9155	2.9171	2.9186	2.9201	2.9217	2.9232	2.9248	2.9263	2.9278
2.32	2.9294	2.9309	2.9325	2.9340	2.9355	2.9371	2.9386	2.9401	2.9417	2.9432
2.33	2.9448	2.9463	2.9478	2.9494	2.9509	2.9524	2.9540	2.9555	2.9570	2.9586
2.34	2.9601	2.9616	2.9632	2.9647	2.9662	2.9678	2.9693	2.9708	2.9724	2.9739
2.35	2.9754	2.9769	2.9785	2.9800	2.9815	2.9831	2.9846	2.9861	2.9876	2.9892
2.36	2.9907	2.9922	2.9938	2.9953	2.9968	2.9983	2.9999	3.0014	3.0029	3.0044
2.37	3.0060	3.0075	3.0090	3.0105	3.0121	3.0136	3.0151	3.0166	3.0182	3.0197
2.38	3.0212	3.0227	3.0242	3.0258	3.0273	3.0288	3.0303	3.0319	3.0334	3.0349
2.39	3.0364	3.0379	3.0395	3.0410	3.0425	3.0440	3.0455	3.0470	3.0486	3.0501
2.40	3.0516	3.0531	3.0546	3.0561	3.0577	3.0592	3.0607	3.0622	3.0637	3.0652
2.41	3.0668	3.0683	3.0698	3.0713	3.0728	3.0743	3.0758	3.0773	3.0789	3.0804
2.42	3.0819	3.0834	3.0849	3.0864	3.0879	3.0894	3.0909	3.0925	3.0940	3.0955
2.43	3.0970	3.0985	3.1000	3.1015	3.1030	3.1045	3.1060	3.1075	3.1090	3.1106
2.44	3.1121	3.1136	3.1151	3.1166	3.1181	3.1196	3.1211	3.1226	3.1241	3.1256
2.45	3.1271	3.1286	3.1301	3.1316	3.1331	3.1346	3.1361	3.1376	3.1391	3.1406
2.46	3.1421	3.1436	3.1451	3.1466	3.1481	3.1496	3.1511	3.1526	3.1541	3.1556
2.47	3.1571	3.1586	3.1601	3.1616	3.1631	3.1646	3.1661	3.1676	3.1691	3.1706
2.48	3.1721	3.1736	3.1751	3.1766	3.1781	3.1796	3.1811	3.1826	3.1841	3.1856
2.49	3.1871	3.1886	3.1901	3.1916	3.1931	3.1945	3.1960	3.1975	3.1990	3.2005
2.50	3.2020	3.2035	3.2050	3.2065	3.2080	3.2095	3.2110	3.2124	3.2139	3.2154

PART II.

FACTORS WHICH INFLUENCE COMPRESSIBILITY, OR ARE CORRELATED WITH COMPRESSIBILITY.

1. ADSORBED WATER.

Wool can adsorb over 30 per cent. of its own weight of water, with an accompanying change in its physical properties. Thus, the resistance of the fibre to both extension and torsion is reduced to a marked degree with the adsorption of water (Speakman, 1927, 1928). Investigations in this direction have been carried out with two objects in view: (1) for comparing the results of tests performed under different conditions of humidity and temperature, and (2) for throwing light on the structure of the fibre. The results have indicated the necessity of performing tests under controlled conditions.

Pidgeon and van Winsen (1934) compressed 3.5 gm. of a sample of dry wool, and 5.0 gm. of a sample of the same wool at 95 per cent. relative humidity. They concluded that "the conditioned sample was less compressible, and showed lower return and compression loops". Larose (1934) compressed four samples of yarn at 50 per cent. and 60 per cent. relative humidities, and corrected for the increase in weight by comparing 3.22 gm. at 50 per cent. with 3.25 gm. at 60 per cent. relative humidity. His results suggested an increase in resistance to compression with an increase in relative humidity.

For the present investigation it was necessary to compare results obtained at 70 per cent. relative humidity with those obtained at 65 per cent. relative humidity, the temperature being 70° F. (21.1° C.) in both cases. For the sake of increased accuracy in evaluating a conversion factor, the range of humidities over which the study was made was extended.

Measurements on the physical properties of wool are entirely satisfactory only when the atmospheric conditions are constant. The "Pendultex" instrument did not permit of determinations under such conditions unless the whole instrument was placed in a room maintained under constant conditions, but in the present investigation an alteration in the conditions of the room employed was not practicable, and the effect of adsorbed water was studied by compressing a sample while containing different amounts of water, and weighing the sample immediately afterwards in order to obtain the amount of adsorbed water.

The method employed was as follows: A 5 gm. sample was exposed to a stream of saturated air for several days, after which it was rapidly placed in the "Pendultex" apparatus and a determination made. On removal it was weighed immediately, teased out, and placed in a jar of which the top could be well sealed. As a result of the exposure during compression and weighing, and the lower initial relative humidity of the atmosphere in the jar (65 per cent.), the sample came into equilibrium with the atmosphere within the jar and contained a smaller amount of adsorbed water. After a week another determination was made and the sample weighed. The procedure was repeated, until the amount of adsorbed water held corresponded to 65 per cent. relative humidity, so that a set of measurements was obtained with the sample containing water from saturation down to 65 per cent. relative humidity. The sample was next dried in a desiccator, and the same procedure carried out in stages from dryness up to 65 per cent. relative humidity.

Finally the dry weight of the sample was determined by heating to a constant weight at 100° C. in the presence of sulphuric acid, under a pressure of 5 cm. Hg. The amount of water held by the sample at each determination was then calculated.

As a result of the repeated determinations, the sample tended to develop numerous small lumps which could not be completely removed owing to the rapidity with which the sample had to be handled. (It has been demonstrated in Part I of this study that the resistance to compression increases for this reason alone.) The difficulty was overcome by exposing a duplicate sample to the constant conditions of the room, and subjecting it to compression each time the test sample was compressed. The duplicate sample showed a gradual increase in resistance to compression with usage, and the results of the test sample were consequently expressed as a ratio of the values obtained for the duplicate sample. The whole procedure was repeated with the two samples interchanged in order to eliminate sampling errors.

Altogether five samples, the constants of which are given in Table 16 (Part I), were utilised for the investigation.

Several factors contributed to the errors of the determinations. In the first place, each figure obtained was the result of one determination only, which had moreover to be performed extremely rapidly. In the second place, the moisture content of the samples may have altered slightly during a determination, and the amount of water held was estimated from the weight obtained after a determination. In the third place, the results were expressed as a ratio of two quantities both subject to error.

At this stage a difficulty presented itself with regard to the interpretation of the results. It has been shown that at constant relative humidity and temperature, the volume occupied by a sample at a given pressure is proportional to the weight of the sample, and samples of different weights can be compared by adjusting the results to correspond to equal weight of the samples. When the same sample is exposed to different values of the relative humidity however, the weight of the sample is altered by an alteration in the amount of water adsorbed. The problem then arises as to whether the results should be compared on the basis of equal amounts of dry wool excluding adsorbed water, or of equal amounts of wool plus adsorbed water.

The former method appears to have been adopted by Larose (1934) for he states: "Another correction which it was necessary to make before results could be compared was that due to the different moisture content of the wool at 50 per cent. and 60 per cent. relative humidities. This difference amounted to about 1 per cent. of the weight of the wool. In order to compare the results obtained at 50 per cent. with those obtained at 60 per cent. humidity it was necessary to subtract 1 per cent. of the values (*of volume*) obtained at 50 per cent. humidity, which is equivalent to comparing 3.25 gm. at 60 per cent with 3.22 gm. at 50 per cent."

The problem will be referred to subsequently, but for the primary object, viz., the comparison of results obtained on 5 gm. of wool at 70 per cent. relative humidity with results obtained on 5 gm. at 65 per cent. relative humidity, the second method was employed, i.e., the results were compared on the basis of equal amounts of wool plus adsorbed water, and the formulae derived in Part I were assumed to hold at all humidities.

In Figure 11 the ratio of the resistance to compression to that of a similar sample with 15.1 per cent. adsorbed water is plotted as a function of the amount of water adsorbed. In Figure 12 the same values are plotted as a function of the corresponding relative humidity under adsorption conditions, as deduced from average values of the amounts of water adsorbed at various relative humidities, previously obtained by the author (van Wyk, 1940). The curves have been completed below 7 per cent. adsorbed water and 30 per cent. relative humidity by eye, and it is not suggested that these portions represent the true courses of the curves. No distinction was made between the five samples in plotting the points, as no systematic difference between them was evident, and the employment of different symbols would merely have reduced the clarity of the figures.

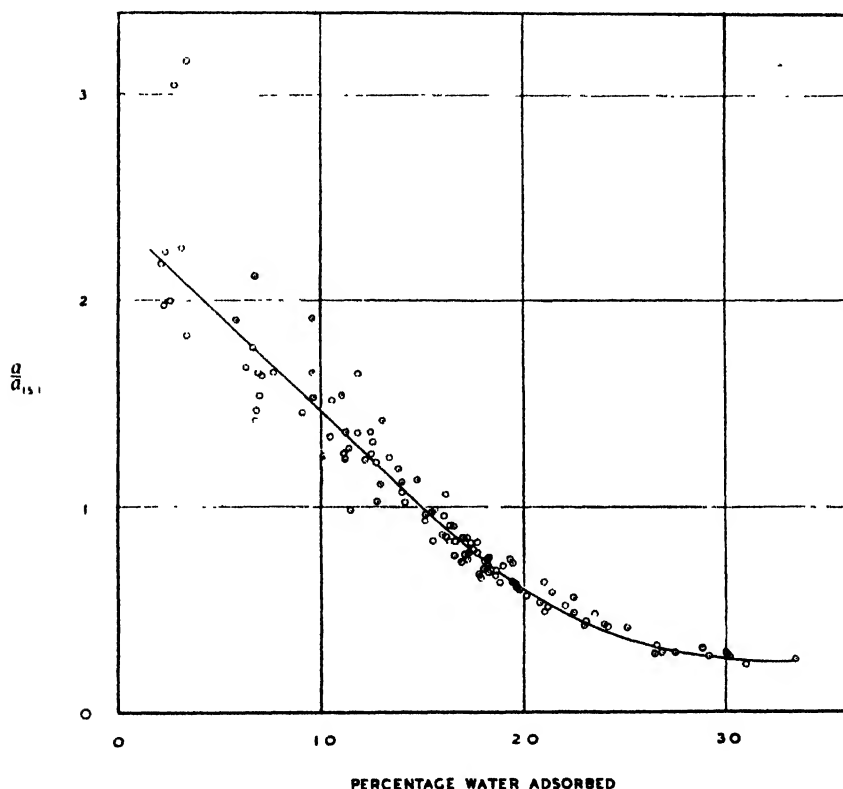


FIGURE 11.—The ratio of the resistance to compression to its value at 15.1 per cent. adsorbed water, as a function of the percentage of water adsorbed (5 samples).

As was to be expected, the points are somewhat scattered and this is especially marked at the low values of adsorbed water, for which several reasons may be advanced. Errors in compressibility determinations have been shown to increase with the resistance to compression. In addition, some of the wools developed such high values of resistance to compression at low values of adsorbed water that they fell outside the range on which the formulae had been based, and the coefficient α had to be estimated by extrapolation. Further, the dry wool was found to adsorb water extremely

rapidly, so that the estimation of the amount of water held was probably subject to a greater error than in the case of higher percentages of water held.

In spite of the scattering of the points the trend is clear. From 7 per cent. to 20 per cent. adsorbed water the relationship may be regarded as linear (Figure 11), and the ratio of the coefficient a at 65 per cent. relative humidity to that at 70 per cent. relative humidity is calculated, by fitting a linear relation, to be 1.122. On the other hand, Figure 12 suggests a linear relation with the relative humidity from 30 per cent. to 100 per cent. Assuming linearity, the ratio is found to be 1.119. It is evident that results obtained at 70 per cent. can be converted to the corresponding value at 65 per cent. relative humidity by simply multiplying by the factor 1.12. All values given in this paper refer to 65 per cent. relative humidity.

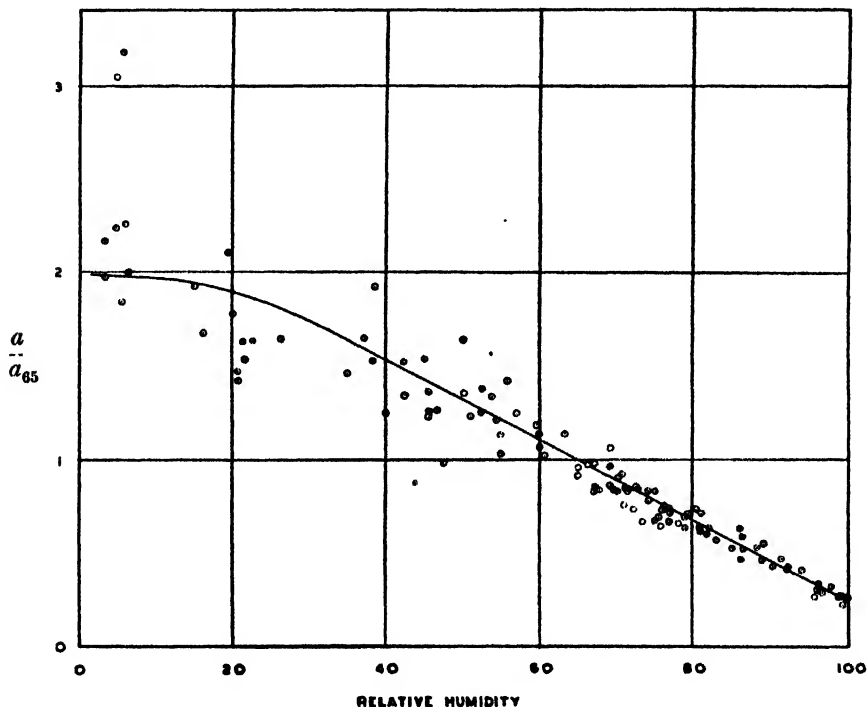


FIGURE 12.—The ratio of the resistance to compression to its value at 65 per cent. relative humidity, as a function of the estimated relative humidity (5 samples).

Besides enabling such a factor to be derived, the results present some features of interest. The curve relating the resistance to compression relative to that at 15.1 per cent. adsorbed water (Figure 11) to the amount of water adsorbed bears a resemblance to the curve illustrating the dependence of the relative rigidity of Cotswold wool on adsorbed water (Speakman, 1928). Speakman found that the adsorbed water at low and high humidities, where adsorption was extremely rapid, had little effect on the rigidity. Figure 11 shows the same tendency at the high values of adsorbed water, the experimental error being too great to allow conclusions to be drawn at the low values of adsorbed water.

Speakman further found that between 5 per cent. and 22 per cent. adsorbed water, the relation between relative rigidity and adsorbed water was linear, and could be expressed by the equation:

$$\text{Relative rigidity} = 1.255 - 0.047 D.$$

where D was the percentage of water adsorbed. According to this formula, the relative rigidity at 15.1 per cent. adsorbed water is 0.545, whence the rigidity relative to 15.1 per cent. adsorbed water is

$$\frac{1.255 - 0.047.D}{0.545} = 2.30 - 0.086.D$$

When a linear equation is fitted to the data illustrated in Figure 11, between 7 per cent. and 20 per cent. adsorbed water, the resistance to compression relative to that at 15.1 per cent. adsorbed water is given by

$$2.34 - 0.089.D, \dots \dots \dots (29)$$

showing a remarkable agreement with Speakman's result.

On the other hand, the linear relation between the ratio and the estimated relative humidity had no counterpart in the case of rigidity, since Speakman found a linear relationship between the logarithm of the reduction in relative rigidity and the logarithm of the relative humidity. In this connection it is to be noted that in the present study the relative humidity was estimated from the amount of water adsorbed, by interpolation of data obtained for other wools.

Discussion.

The question has been raised as to whether the values of resistance to compression offered by the same sample when containing different amounts of adsorbed water should be compared on the basis of equal amounts of dry wool, or of equal amounts of wool plus adsorbed water. The latter method has been employed in the present study, with the results shown in Figure 11.

From dryness to saturation, the mass of a fibre increases by about 33 per cent., the area of cross-section by about 32 per cent., the length by 1.2 per cent., while the specific gravity at first rises to a maximum and then decreases to saturation (Hirst, 1922; King, 1926; Speakman, 1928, 1930). In the case of King's determination, the specific gravity was 1.304 dry and 1.265 at saturation, a difference of 3 per cent. It is to be noted that the increase in volume is almost entirely due to lateral swelling of the fibre, and that the change in specific gravity is small compared to the changes in mass and volume.

A comparison on the basis of equal amounts of dry wool may thus in practice be regarded as equivalent to a comparison of equal total lengths of fibre. When considering the comparison of different wools at the same relative humidity (Part I), it was stated that the only difference between the comparison of equal masses and the comparison of equal lengths of fibre lay in some power, probably the sixth, of the respective fibre diameters. A similar argument is applicable to the same sample at different values of the relative humidity, for the changes in length and specific gravity may be considered negligible.

Taking Hirst's (1922) results for the swelling of an English wool fibre, and multiplying the resistance to compression by the sixth power of the relative diameter, an approximate relation for the resistance to compression relative to 15.1 per cent. adsorbed water is

$$1.75 - 0.050.D$$

where D is the percentage of water adsorbed.

Speakman (1930) gives the value of Young's modulus by stretching Cotswold wool fibres at different values of adsorbed water. A rough calculation gives for the Young's modulus relative to 15.1 per cent. adsorbed water:

$$1.41 - 0.027.D$$

Thus, even when the resistance to compression is adjusted to correspond to equal lengths of fibre, i.e., equal masses of wool excluding adsorbed water, the change with adsorbed water is still about twice that of Young's modulus obtained by stretching the fibre.

An interesting consideration is provided by the results of Pidgeon and van Winsen (1934). These authors give the pressure-volume relations of 3.5 gm. of a dry sample, and of 5.0 gm. of the same sample at 95 per cent. relative humidity. In Part I of this paper it was shown that the equation

$$p = Pe^{\frac{Qm}{(v-v')}} - R \dots \dots \dots (11)$$

fitted experimental results closely. On applying this equation to the above-mentioned data of Pidgeon and van Winsen, the values of the product Qm were found to be 44.8 for the dry sample and 69.5 for the conditioned sample. Assuming the dry weight of the conditioned 5 gm. sample at 95 per cent. relative humidity to have been 4.0 gm., the values of Q corresponding to both methods of comparison may be calculated, as shown in Table 21.

TABLE 21.

The coefficient Q calculated on the basis of equal masses of dry wool and on the basis of equal masses of wool plus adsorbed water, from data by Pidgeon and van Winsen.

Basis of Comparison.	Dry Sample. $Qm = 44.8$.		Conditioned Sample. $Qm = 69.5$.	
	m	Q	m	Q
Equal masses of dry wool.....	3.5	12.8	4.0	17.4
Equal masses of wool plus absorbed water.	3.5	12.8	5.0	13.9

When the comparison is based on equal masses of wool plus water, the two values of Q show closer agreement than when equal masses of dry wool are considered.

The possibility must, however, be considered that the coefficient Q is a function of the fibre diameter. Now the values obtained in the present study were higher, for finer wools, than those of Larose, suggesting that an increase in Q associated with a decrease in fibre diameter, whereas in Table 21 a higher value for Q is obtained for the swollen fibre.

On the other hand, assuming that differences in Q previously obtained were due to causes other than differences in fibre diameter, the coefficient Q may be supposed, for example, to include the cross-sectional area as a factor. The value of 17.4 obtained in Table 21 from the dry weight of the conditioned sample may be reduced to 13.4 on the assumption that the increase in cross-sectional area is 30 per cent. from dryness to 95 per cent. relative humidity.

In view of the uncertainty as to the exact rôle played by fibre diameter, such considerations must be regarded only as interesting possibilities.

Larose (1934), comparing his results on the basis of equal amounts of dry wool, found an increase in resistance to compression with an increase in relative humidity. Such a conclusion is hardly acceptable in view of the reduction in the resistance of the fibre to both extension and torsion with the adsorption of water. On the other hand, it should be borne in mind that Larose's determinations were made on yarn, and it is probable that the swelling of the fibres may cause a stiffening of the yarn. At extremely high pressures, an increase in the volume will result from the increase in the volume of the fibres themselves with adsorption of water. Such high pressures have not, however, been employed, except possibly in Burns and Johnston's (1936) yield determinations.

Finally, the author cannot regard the agreement of his results with those of Speakman's (1928) rigidity determinations as a mere coincidence, and on the whole considers that there is considerable justification for the method of comparing results at different humidities on the basis of equal amounts of wool plus adsorbed water, no correction being applied for the alteration in fibre diameter.

2. DIMENSIONAL ATTRIBUTES OF THE FIBRE.

The fibre attributes of length, fineness and crimping are the most readily estimated characteristics of a wool sample, and together form the main basis of practical wool classification.

(a) *Length.*

During the same period of growth the fleeces grown by Merino sheep vary considerably in fibre length and staple length, and in order to compare the compressibility of different wools it is necessary, from a purely experimental point of view, that the effect of length should be determined, quite apart from its importance to both producer and manufacturer.

Winson (1932) stated that on the whole the "resilience" of a sample (i.e., the area of the loop enclosed between the compression and release curves) was increased when the fibre length was reduced. Henning (1934) found very little difference in the number of swings recorded by the "Pendultex" instrument for fibres shorter than 40 mm. and those longer than 50 mm. in the same top.

The determination of the effect of length on compressibility is likely to be influenced by two factors. In the first place, the ratio of the straight fibre length to the crimped or staple length varies for different fleeces, and secondly, the effect of length may be disturbed by other properties associated with the rate of growth of the fibres. The obvious method of overcoming these factors is to employ different lengths of the same staple.

With this end in view, use was made of a fleece of approximately 10 inch (25 cm.) staple length, grown by a sheep which had not been shorn for 28 months. Small but definite variations in the crimping along the length of the staple were visible, pointing to variations in fibre thickness, such as are produced by changes in the health or nutrition of the sheep. A careful system of sampling had, therefore, to be employed in order to eliminate the possible effect of other factors such as fibre thickness and crimping.

Several staples were selected and cut to a length of 20 cm. by removal of the tip ends, variations due to weathering of the tips being thus minimised. Each staple was separated into ten portions as nearly equal as could be judged by eye, and ten sub-samples were made up, each consisting of one such portion from each of the original staples.

The ten sub-samples were next graded down in 2 cm. intervals from 20 cm. to 2 cm., care being taken to ensure that each final sample was composed of portions taken along the entire length of the staple. For example, the 18 cm. sample was obtained by cutting off in succession a 1 cm. length from each end of the first portion, a 2 cm. length from the tip end of the next portion, and a 2 cm. length from the root end of the following portion. A similar procedure was adopted for obtaining the other lengths, and as a check on the adequacy of the sampling technique, the mean fibre thickness of each final sample was determined.

TABLE 22.

The effect of length on the resistance to compression.

Sample.	Staple Length. (cm.).	Resistance to Compression. (a) (Kg. cm. ² per 5 gm.)	Fibre Thickness. (microns).
1.....	2	5.4×10^3	23.7
	4	5.7×10^3	23.4
	6	5.6×10^3	23.3
	8	5.6×10^3	23.5
	10	5.9×10^3	23.5
	12	5.8×10^3	23.5
	14	5.7×10^3	23.4
	16	5.6×10^3	23.0
	18	5.7×10^3	23.3
	20	5.8×10^3	23.9
2 (composite).....	2	6.4×10^3	23.3
	4	6.7×10^3	23.4
	6	7.0×10^3	23.1
	8	6.8×10^3	23.2
3.....	2.5	6.2×10^3	25.4
	5.0	6.0×10^3	25.4
	7.5	6.5×10^3	25.5
	10.0	6.3×10^3	25.2
4 (crossbred).....	2.5	4.5×10^3	41.0
	5.0	5.0×10^3	41.0
	7.5	5.2×10^3	40.6
	10.0	5.0×10^3	42.3
	12.5	5.2×10^3	41.2

Three other samples of staple lengths up to 12.5 cm. were procured and subjected to a similar procedure. The resistance to compression was determined by the "Pendultex" method, and the whole procedure was carried out in duplicate. The results are given in Table 22.

As shown by the mean fibre thickness, the system of sampling can be regarded as having been adequate.

The results point to the conclusion that length has no measurable influence on the resistance to compression as determined by the dynamic method, down to staple lengths of about 2.5 cm. or one inch. At this value the coefficient shows a tendency to drop.

The independence of resistance to compression on length, for lengths above a certain value, greatly facilitates determinations on compressibility, obviating as it does the tedious process of cutting all staples to a certain length, or of correcting for the length. Even the procedure of cutting all the staples to the same length would be no guarantee of the equality of the straight fibre lengths, owing to the large differences occurring among wools in the ratio of the straight to the crimped fibre length (Duerden and Bosman, 1931). In the present study errors due to this cause were eliminated by employing staples grown on adjacent areas of the skin, and by an adequate system of sampling.

It is to be emphasised that no comparison has been made between the compressional characteristics of the so-called "quick-growing" and "slow-growing" wools. Where differences between such wools are obtained, it is safe to assume that they are not due to differences in the length itself, but to other factors associated with the rate of growth.

(b) *Fibre thickness.**

The average fibre thickness is accepted as being the most important single property determining the spinning count and quality number of wool, and the relationship between quality number and fibre thickness has consequently been investigated to a considerable extent. Among standards which have been compiled, those of Duerden (1929) are of direct interest to South African wool production. Quality appellations in different countries have been compared (Schneider, 1929; Winson, 1931) with the object of standardising tops internationally on a fineness basis. It is evident, therefore, that in the study of any wool attribute, its relation to, or dependence on, fibre thickness assumes considerable importance.

In Part I of this study, compression of the fibre mass was considered as the bending of fibre elements between adjacent contacts. In a given mass of wool, the total length of fibre is reduced by an increase in the fibre thickness, and there is a consequent reduction in the number of contacts and the number of elements undergoing bending, and a corresponding increase in the mean length of the elements. On this score alone, the effect of an increase

* In the present paper, the term "fibre thickness" has been generally adopted. Objection has been made to the use of the term "diameter" on account of the non-circularity of the fibre cross-section. The term "fineness" is the one most widely adopted in wool practice, but as it may, strictly speaking, be regarded as the reciprocal of thickness, its use in such expressions as "an increase in fineness" may lead to confusion, and in the present paper it is employed only in a general sense, or where the work of other authors is being quoted.

in fibre thickness is a reduction in resistance to compressions. On the other hand, the force necessary to bend a fibre by a certain amount increases with the thickness, so that the fibre thickness has two opposing effects on the resistance to compression. In the theoretical discussion (Part 1) three cases were considered where the resistance to compression depended on the mass of material and was independent of the diameter.

The only study thus far recorded of the effect of fibre thickness is that of Henning (1934), who determined the number of swings recorded by the "Pendultex" instrument while compressing tops of different qualities. He concluded that "with diminishing fineness (i.e., increasing fibre thickness), the resistance to compression (*Bauschigkeit*) at first improves, but falls on passing over to the long coarse wools".

In the present study it was a matter of routine to determine the mean fibre thickness of each sample tested for resistance to compression. Before washing the sample, a small strand was removed from each staple, and the strands were grouped together to form a bundle. The technique subsequently followed has been fully described elsewhere (Bosman and van Wyk, 1939). At least 500 measurements were made on a sample, initially with a Zeiss-Hegener Micro-camera (1 division = 2.5μ) and later with a Zeiss Lanameter (1 division = 2μ).

It will be shown later that the effect of fibre thickness cannot be considered alone in practice, since other factors especially crimping, complicate the effect. It is to be noted, however, that the total correlation coefficient between resistance to compression and fibre thickness was found to be -0.0065 for 310 samples from various sources. This completely insignificant value leads to the conclusion that among Merino wools generally no correlation exists between the two attributes.

Thus the experimental result appears to agree with the theoretical expectation, but it cannot be accepted as proof that in practice the fibre thickness has no influence on the resistance to compression, for other factors correlated with the fibre thickness may oppose its effect. Such a factor is the crimping, to be considered in the following section.

(c) *Crimping.*

The crimping, or wave form, of the fibre has been the subject of a number of researches from different aspects. The origin of the crimping was attributed by Bowman (1908) to unequal contraction of the cells on the two sides of the fibre, while Wildman (1931) found evidence of a rotation of the bulb of the follicle. Such a rotation could account for the presence of twist in the fibre, shown by Rossouw (1931) and Woods (1935) to have a periodic reversal corresponding to the crests and troughs of the crimp waves. Barker and Norris (1930) postulated that "the crimp of wool fibres can be accounted for by hypothesising two periodic or simple harmonic forces acting at right angles at the follicle, in addition to the force exerted to promote extrusion and growth".

The work of Norris and van Rensburg (1930) and Norris and Claassens (1931) suggested that crimp formation was a periodic function of time and independent of the rate of growth of the fibre. This conclusion was not, however, supported by the work of Swart and Kotzé (1937).

The relation between the number of crimps per unit length of fibre and the fibre thickness has received the attention of several research workers, and conflicting results have been obtained. In considering the results obtained by various authors, one is led to the conclusion that among Merino wools generally a negative correlation of the order of -0.5 exists between the two quantities, but within certain groups no correlation or even a small positive correlation may be found. Data on South African Merino wools have been given by Duerden (1929), Duerden and Bell (1931), Bosman and Botha (1933), Bosman (1937:1), Reimers and Swart (1929, 1931) and Swart (1937). The importance of the relation between the number of crimps per unit length and the fibre thickness lies in the fact that the crimping is usually taken as the main basis for estimating the fibre thickness in practical wool classification.

By analogy with the bending of a strut, Barker and Norris (1930) predicted that for fibres of circular cross-section, and the same value of Young's modulus, the product of the number of crimps per unit length and the square of the fibre diameter should be constant. They verified this conclusion experimentally in a few cases, and showed that Duerden's (1929) results followed the law when allowance was made for the ellipticity of the fibre cross-section.

The idea that the crimping is associated with the elastic properties of the fibres appears to be current among woolmen. For example, van der Merwe (1926) states that "a fine wool, being more numerously crimped than a strong wool, shows greater elasticity". Duerden (1929) stated that "it is hoped to show later that the crimps may be regarded as a measure of pliability". On the manufacturing side Speakman (1937) states that "the waviness or crimpiness of wool, such as Merino wool, is a very valuable property, a characteristic loftiness and sponginess of handle being thereby produced in the fabric".

The only direct investigation appears to be that of Henning (1934), who found that partial removal of the crimp by dyeing reduced the resistance to compression.

For a complete study of the effect of crimping on the compressibility of the fibre mass it is necessary that both the length and the depth of the wave should be taken into account. In the present investigation the length only has been considered, partly on account of the labour involved in measuring, on a routine basis, the shape of the crimp wave, necessitating as it does the measurement of large numbers of individual fibres or strands (Wildman, 1939), and partly because the present study was restricted mainly to the more obvious and readily estimated properties of the wool. In this connection it is to be noted that Wildman (1939) found that the ratio of the straight to the crimped fibre length was not a reliable index of the depth of the wave.

In the present study, the number of crimps was estimated by setting the points of a pair of dividers exactly an inch apart and counting the number of complete waves between the points; either crests or troughs were counted, and not both, as some authors appear to have done. Owing to the variability within a sample, measurements were made on all the staples occurring in a sample, so that the figure obtained for each sample was the mean of from 50 to 100 measurements. It should be noted that the crimp measurements were made on a greasy wool, while the compressibility tests were made subsequent to immersion in water. This question will be referred to later.

The total correlation coefficient between the resistance to compression as defined and the number of crimps per inch was found to be +0.5533 for the 310 samples tested. This highly significant value shows that the resistance to compression increases as the number of crimps per inch increases.

Moreover, with the correlation coefficient of -0.5544 between the number of crimps per inch and the fibre thickness, it was possible to calculate the partial correlation coefficients for the two attributes separately, as shown in Table 23.

TABLE 23. *

The correlation with resistance to compression.

Attribute.	Total.	Partial.
Fibre thickness.....	-0.0065	+0.4330
Number of crimps per inch.....	+0.5533	+0.6604

The partial correlation coefficient between resistance to compression and fibre thickness is highly significant at the 1 per cent. probability level, suggesting that the fibre thickness has a positive influence on the resistance to compression, but that the effect is masked by the crimping, which is negatively correlated with the fibre thickness.

The two partial correlation coefficients suggest that in experimental work it should be possible to determine to what extent differences in compressibility may be accounted for by differences in fibre thickness and crimping. For this purpose it is necessary to know the relationship existing between the three quantities. Even after the effect of fibre thickness and crimping had been taken into account, however, the variability in compressibility was so great as to preclude the possibility of deriving the exact relationship from the experimental data. This was obviously due to the influence of other factors which had not been taken into account. Nor was it found possible to derive the relationship theoretically. In such a case it was thought justifiable to employ a linear relation, expressible by the equation

$$a = 357.d + 623.n - 6919 \dots \dots \dots (30)$$

where a was the resistance to compression as defined, d the mean fibre thickness in microns, and n the number of crimps per inch of staple. The equation corresponds to that commonly employed in co-variance analysis where linearity between the variables is assumed.

Equation (30) is not the only suitable one. When the logarithms of the variables are assumed to bear a linear relation to one another, the coefficients of $\log d$ and $\log n$ are found to be 0.98 and 0.94 respectively. These values are so nearly equal to unity as to suggest that the resistance to compression may be taken to bear a linear relation to the product nd , giving

$$a = 2952.nd + 694 \dots \dots \dots (31)$$

The efficacy of equations (30) and (31) in removing variability from a is illustrated by the analysis of variance in Table 24.

TABLE 24.

Analysis of variance in resistance to compression.

Variance due to —	EQUATION (30).			EQUATION (31).		
	D.F.	Standard Deviation. (Kg. cm. ² per 5 gm.).	z	D.F.	Standard Deviation. (Kg. cm. ² per 5 gm.).	z
Regression.....	2	15.58×10^3	2.389	1	22.26×10^3	2.755
Error.....	307	1.430×10^3		308	1.416×10^3	
TOTAL.....	309	1.898×10^3		309	1.898×10^3	

In both cases the value of z , i.e., the natural logarithm of the ratio of the two standard deviations, is highly significant at the 1 per cent. probability level. Judging by the residual variation, there appears to be little to choose between the two equations, and when the equations were later applied to co-variance analysis, no difference between the results was evident. It is also clear that only a portion of the variability in resistance to compression can be ascribed to fibre thickness and crimping. As an illustration of the residual variation, the coefficient a is plotted as a function of the product nd in Figure 13.

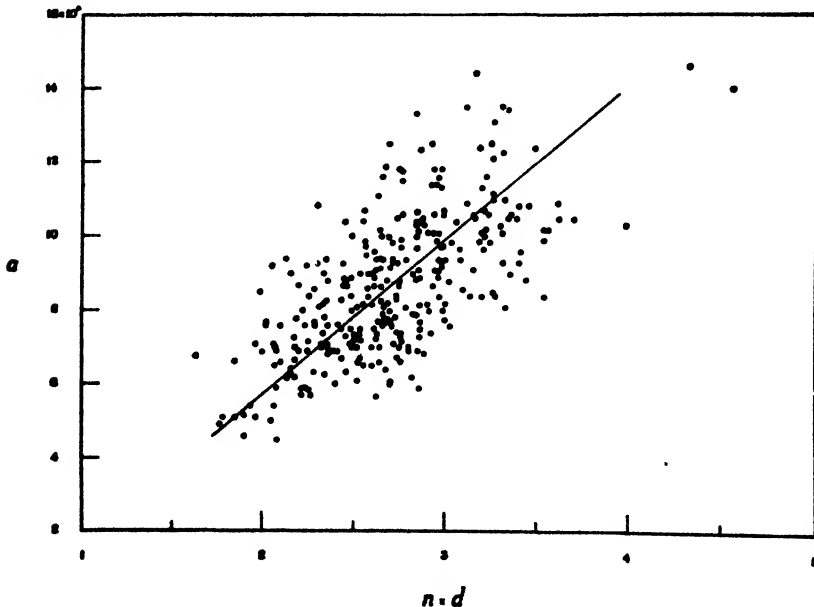


FIGURE 13.— The resistance to compression a plotted as a function of the product of the number of crimps per inch n and the fibre thickness d .

The standards of fibre thickness and crimping compiled by Duerden (1929) may be utilised for comparing the relative effects of the two factors on the compressibility. From equation (30), the value of a for a typical

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58's wool with $d=24.25\mu$ and $n=8.5$ is 7.0×10^3 Kg. cm.² per 5 gm. If the crimps remain the same, and the fibre thickness is reduced to 22.15μ to correspond to a 60's wool, the coefficient a will be reduced by 11 per cent. If on the other hand, the thickness remains the same, and the number of crimps per inch is increased to 10.5 to correspond to a 60's wool, the coefficient a will be increased by 18 per cent. For a 66's wool, the effect of reducing the thickness to correspond to a 70's reduces a by 4 per cent., while increasing n to correspond to a 70's increases a by 14 per cent. For both wools it is evident that an alteration in the crimping by one quality number class produces a greater effect on the resistance to compression than a corresponding alteration in the fibre thickness. The greater difference between the two effects in the case of the fine wool is due to the fact that while the difference between the classes is nearly constant as regards the number of crimps per inch, the difference in fibre thickness between the classes diminishes rapidly as the wool becomes finer.

In a previous communication (van Wyk, 1939) it was suggested that wool samples which are finer than the crimps indicate have a lower resistance to compression than wools which are coarser than the crimps indicate. For testing this statement, the standards of Duerden were again utilised. The 310 samples were classed according to both fibre thickness and crimping, and the differences between the classes correlated with the resistance to compression. The correlation is illustrated in Table 25, where a positive value of the difference means that the wool is coarser than the crimps indicate, and a negative value that the wool is finer than the crimps indicate.

TABLE 25.

The correlation between the resistance to compression and the difference between the classes as given by fibre thickness and crimping according to Duerden's standards (310 samples).

Resistance to Compression. (Kg. cm. ⁷ per 5 gm.).	DIFFERENCE BETWEEN CLASSES.									
	-4	-3	-2	-1	0	+1	+2	+3	+4	
4- 5 × 10 ³	—	—	1	2	—	—	—	—	—	
5- 6 × 10 ³	1	—	2	1	7	5	—	—	—	
6- 7 × 10 ³	—	—	2	9	14	9	3	—	—	
7- 8 × 10 ³	1	—	2	15	14	27	5	1	—	
8- 9 × 10 ³	—	—	—	5	14	21	14	1	—	
9-10 × 10 ³	—	—	—	2	13	23	11	5	—	
10-11 × 10 ³	—	—	—	3	6	9	17	11	1	
11-12 × 10 ³	—	—	—	2	1	5	7	2	—	
12-13 × 10 ³	—	—	—	1	—	—	5	2	—	
13-14 × 10 ³	—	—	—	—	—	—	4	1	—	
14-15 × 10 ³	—	—	—	—	—	—	1	1	1	

$$r = +0.5174.$$

The correlation coefficient of +0.5174 is highly significant at the 1 per cent. probability level, and the statement that wools which are finer than the crimps indicate have a lower resistance to compression than wools which are coarser than the crimps indicate may be regarded as justified, when Duerden's standards are taken as criterion. In view, however, of the

criticism to which the standards have been subjected, the question was also viewed differently. Instead of considering differences according to Duerden's standards, deviations of fibre thickness from an average relation between fibre thickness and crimping of the 310 samples under consideration were correlated with the resistance to compression. Such a relation is given by

$$\text{Log}_{10} d = 1.6716 - 0.3069 \cdot \log_{10} n.$$

This equation was obtained by regarding $\log n$ as the independent variable and $\log d$ as the dependent variable, since the point at issue was the deviation of fibre thickness from its value as estimated from the crimping. The correlation is illustrated in Table 26.

TABLE 26.

The correlation between resistance to compression and the deviation of fibre thickness from its value as estimated from the crimping, by means of the average relation for the 310 samples.

Resistance to Compression (Kg. cm. ² per 5 gm.).	Deviation of fibre thickness from its value as estimated (microns).										
	-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5
4-5 $\times 10^3$	—	1	—	2	—	—	—	—	—	—	—
5-6 $\times 10^3$	1	2	—	4	3	3	2	1	—	—	—
6-7 $\times 10^3$	—	2	5	5	10	9	2	1	—	—	—
7-8 $\times 10^3$	1	—	6	11	13	18	6	6	4	—	—
8-9 $\times 10^3$	—	—	1	6	14	13	8	9	4	—	—
9-10 $\times 10^3$	—	—	1	6	13	14	7	5	3	5	—
10-11 $\times 10^3$	—	—	—	7	4	9	10	8	4	4	1
11-12 $\times 10^3$	—	—	—	3	2	4	3	2	2	1	—
12-13 $\times 10^3$	—	—	—	1	—	—	3	3	—	1	—
13-14 $\times 10^3$	—	—	—	—	—	2	—	2	1	—	—
14-15 $\times 10^3$	—	—	—	—	—	—	1	1	—	1	—

$$r = +0.3665.$$

The coefficient of +0.3665 is somewhat smaller than that found from Table 25, but it is also highly significant at the 1 per cent. probability level, and leads to the same conclusion, viz., that wools which are finer than the crimps indicate have a lower resistance to compression than wools which are coarser than the crimps indicate.

Since the number of crimps per inch and the fibre thickness both have a positive effect on the resistance to compression, and the number of crimps per inch increases while the fibre thickness diminishes with the quality number, it is of interest to determine the relation between the resistance to compression and the quality number. For this purpose Duerden's standards have been employed, and they are reproduced in the first three columns of Table 27. In the fourth column are given the values of the resistance to compression as calculated by means of equation (30) from the mean thickness and the mean number of crimps per inch in each class. In columns 5 and 6 the averages of the values actually obtained when grouped according to crimping are shown together with the frequency within each class. The last two columns contain the averages of the values obtained in the thickness groups, and the frequency within each group. The smaller number of

observations in the crimp groups is due to the fact that samples with crimps intermediate between the classes have been excluded in order that the means might be directly comparable with the calculated values.

TABLE 27.

The variation of resistance to compression with quality number.

Duerden's Standards.			Resistance to Compression as calculated from Equation (30). (Kg. cm. ⁷ per 5 gm.).	Determined Resistance to Compression (Kg. cm. ⁷ per 5 gm.) for samples grouped according to—			
Quality No.	Crimps per Inch.	Fibre thickness (Microns).		Crimps.		Thickness.	
				Mean.	Frequency.	Mean.	Frequency.
100's	22-24	15.4-16.2	13.0 × 10 ³	14.0 × 10 ³	1	9.1 × 10 ³	1
90's	20-21	16.2-17.0	11.8 × 10 ³	9.7 × 10 ³	2	7.6 × 10 ³	1
80's	18-19	17.0-17.9	10.8 × 10 ³	11.2 × 10 ³	3	9.1 × 10 ³	3
70's	16-17	17.9-18.9	9.9 × 10 ³	10.3 × 10 ³	12	9.1 × 10 ³	22
66's	14-15	18.9-20.0	9.1 × 10 ³	9.0 × 10 ³	24	8.6 × 10 ³	48
64's	12-13	20.0-21.3	8.2 × 10 ³	8.5 × 10 ³	61	8.6 × 10 ³	59
60's	10-11	21.3-23.0	7.5 × 10 ³	7.7 × 10 ³	52	8.5 × 10 ³	94
58's	8-9	23.0-25.5	7.0 × 10 ³	6.7 × 10 ³	10	9.2 × 10 ³	54
56's	6-7	25.5-29.0	6.9 × 10 ³	5.1 × 10 ³	1	8.4 × 10 ³	28
					166		310

The values calculated by means of equation (30) from the mean thickness and the number of crimps in each class show an increase with the quality number. As is to be expected from the total correlation coefficients (Table 23), the means of the values when grouped according to the crimping also show an increase with the quality number, while the means of the values grouped according to fibre thickness appear to be constant and independent of the quality number. Thus, while it is true that no general relationship has been found between the resistance to compression and the fibre thickness, the practical estimation of quality number is based mainly on the crimping, and it may be concluded that in general the resistance to compression will increase with the quality number.

(d) Variability in fibre thickness.

It has been shown that fibre thicknesses within a sample are so distributed that the logarithm of fibre thickness follows the normal law of distribution (Malan, 1937; Malan, Carter and van Wyk, 1938). A correlation exists between the mean and the standard deviation, and no correlation between the mean and the coefficient of variability (Bosman, 1937:1).

Where the breeder aims at uniformity in the fleece, the variability assumes almost as much importance as the mean fibre thickness, and it is of interest to determine whether compressibility is associated with the fibre variability. To this end the coefficients of correlation with compressibility were calculated for both the standard deviation and the coefficient of variability in fibre thickness, with the results given in Table 28.

TABLE 28.

Coefficient of correlation between resistance to compression and variability in fibre thickness (310 samples).

	Resistance to Compression.	Fibre thick- ness.	Crimps per Inch.	Standard Deviation.	Coefficient of Variability.
Resistance to compression.	--	--0.0065	0.5533	0.0332 (T) + 0.1518 (P)	+ 0.0568 (T) + 0.1578 (P)
Fibre thickness.....	-- 0.0065	--	0.5544	0.5968	-- 0.1054
Crimps per inch.....	0.5533	-- 0.5544	--	0.3988	-- 0.0187
Standard deviation.....	+ 0.0332 (T) + 0.1518 (P)	0.5968	-- 0.3988		--
Coefficient of variability...	+ 0.0568 (T) + 0.1578 (P)	-- 0.1054	0.0187	--	--

In the case of the standard deviation and coefficient of variability, the partial coefficients, obtained by eliminating the effects of fibre thickness and crimping, are also given and designated (P).

Applying the *t* test (Fisher, 1932) the two total correlation coefficients are shown to be completely insignificant, while the probability of obtaining the two partial coefficients from an uncorrelated population is just below 1 per cent. The partial coefficients may, therefore be regarded as significant, but they are so small that it is doubtful whether their influence need be considered in practice.

In agreement with the results of Bosman (1937:1), no correlation was found between mean fibre thickness and the coefficient of variability, while a highly significant correlation coefficient of +0.5698 was obtained between mean fibre thickness and standard deviation.

3. SURFACE FRICTION.

The compression of a mass of fibres will be accompanied by a tendency on the part of the fibres to slip over one another, and Pidgeon and van Winsen (1934) even go so far as to say that "the pressure-volume relation of a mass of fibres is ultimately dependent on the ease with which they slip over one another". It is highly probable that one of the factors which influence the slippage of the fibres is the surface friction, determined in wool largely by the surface scales.

Taking another point of view, Matthews (1904) states: "The rigidity and pliability of the wool fibre is also largely conditioned by the nature of epidermal scales. If these fit over one another loosely with considerable length of free edge, the fibre will be very pliable and plastic, soft and yielding, also easily felted. Whereas, if the scales fit closely against one another and have little or no freedom of movement, the fibres will be stiff and resistant, and not easily twisted together nor felted".

Since the surface scales point in the direction of the tip of the fibre, the friction is greater when the fibre is travelling in the direction of the tip than when it is travelling in the direction of the root. Whether the fibres tend towards a uni-directional motion during compression has not been ascertained, and it is therefore a difficult matter to decide what combination of the two coefficients of friction will be the most likely to influence the compressibility. Such uni-directional motion takes place during the felting process, so that Speakman and Stott (1931) employed the percentage difference between the two coefficients of friction in their studies on milling.

In the present study, the coefficients of friction of 94 samples in the two directions of the fibre were determined by the method described by Bosman and van Wyk (1941), and the resistance to compression of the samples was determined by means of the "Pendultex" apparatus.

The following symbols were employed, and the correlation coefficients are given in Table 29.

- s_1 — coefficient of friction of fibres moving in direction of tip,
 s_2 — coefficient of friction of fibres moving in direction of root,
 $s_1 - s_2$ — difference between coefficients of friction,
 S = percentage difference between coefficients of friction.

$$= \frac{s_1 - s_2}{s_2} \times 100,$$
 $\frac{1}{2} (s_1 + s_2)$ = mean coefficient of friction,
 d = mean fibre thickness,
 n = number of crimps per inch,
 a = coefficient of resistance to compression.

TABLE 29.

The correlation coefficients between resistance to compression, mean fibre thickness, number of crimps per inch, and the coefficients of surface friction of 94 samples.

	a	d	n	s_1	s_2	$s_1 - s_2$	S	$\frac{1}{2} (s_1 + s_2)$
a	—	+0.0803	+0.5286	+0.0489	+0.0543	-0.0576	-0.1703	+0.0934
d	+0.0803	—	-0.4987	-0.2645	-0.0616	-0.2588	-0.1918	-0.2506
n	+0.5286	-0.4987	—	+0.0685	+0.0539	-0.0261	-0.1214	+0.1065
s_1	+0.0489	-0.2645	+0.0685	—	+0.2636	—	—	—
s_2	+0.0543	-0.0616	+0.0539	+0.2636	—	—	—	—
$s_1 - s_2$	-0.0576	-0.2588	-0.0261	—	—	—	—	—
S	-0.1703	-0.1918	-0.1214	—	—	—	—	—
$\frac{1}{2} (s_1 + s_2)$	+0.0934	-0.2506	+0.1065	—	—	—	—	—

The coefficients show that no correlation exists between the resistance to compression and the surface friction of the fibres. When the effects of fibre thickness and crimping are eliminated, the coefficients shown in Table 30 are obtained.

TABLE 30.

The total correlation coefficients between resistance to compression and surface friction, and the partial coefficients obtained after eliminating the effects of fibre thickness and crimping.

Resistance to Compression and -		Correlation Coefficients.	
		Total	Partial.
s_1	+0.0489	+0.1519
s_2	+0.0543	+0.0557
$s_1 - s_2$	-0.0576	+0.1133
δ	-0.1703	+0.0133
$\frac{1}{2} (s_1 + s_2)$	+0.0934	+0.1757

For the number of samples, a coefficient has to exceed 0.24 in order to be regarded as significant at the 5 per cent. probability level. Since all the coefficients are smaller than this value, they must be regarded as insignificant, and it may be concluded that no relation exists between the compressibility of a wool sample and the surface friction of its component fibres.

4. TENSILE STRENGTH.

The tensile strength of a sample of wool is generally taken as an indication of the soundness of the wool. It is estimated by hand when wool is judged, and on the experimental side it has been the most widely investigated of all the mechanical properties of the wool fibre. Since the resistance to compression is a measure of the elastic properties of wool in bulk, it is of interest to investigate a possible relation between the two properties.

For this purpose the tensile strength of 130 samples was determined by means of bundle tests, the method employed being that described by Bosman, Waterston and van Wyk (1940), while the resistance to compression of the same samples was determined with the "Pendultex" apparatus.

For the 130 samples the total correlation coefficient between resistance to compression and tensile strength was found to be -0.0098, a completely insignificant value. After elimination of the effects of fibre thickness and crimping, the coefficient was +0.1491, which is still insignificant at the 5 per cent. probability level. It must be concluded, either that the resistance to compression is not associated with the tensile strength, or that other factors influence one of these characteristics and not the other, thus masking a possible correlation.

5. SPECIFIC GRAVITY.

Van Wyk and Nel (1940), have pointed out that in spite of experimental evidence to show that the specific gravity of different wools varied but slightly, there was a belief among woolmen that marked variations occurred among different types of Merino wool. Thus, Hawkesworth (1920) regards a high "density of fibre" as desirable, while Cowley (1928) associates "density of fibre" with fineness. Provision is also made for the specific gravity in some South African wool score-cards.

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In the paper quoted above, the results of determinations on samples selected for specific gravity by a leading sheep and wool expert were recorded, and it was concluded that the evidence was inconclusive, and that the samples had been selected for some other property assumed to be, or to be associated with, specific gravity.

The resistance to compression of the samples given in Tables 4 and 5 of the paper quoted, was determined, and the results are given in Table 31, together with the specific gravities.

TABLE 31.

The specific gravity and resistance to compression of two groups of four samples each, selected for specific gravity.

Group.	Sample.	Specific Gravity.	Resistance to Compression. (Kg. cm. ² per 5 gm.).
1	1 presumed higher S.G.....	1.301	13.7×10^3
	2	1.301	13.5×10^3
	3	1.303	8.4×10^3
	4 presumed lower S.G.....	1.301	7.2×10^3
2	1 presumed higher S.G.....	1.303	11.7×10^3
	2	1.303	8.9×10^3
	3	1.298	7.6×10^3
	4 presumed lower S.G.....	1.301	5.8×10^3

It is evident from the table that the sheep and wool expert concerned selected the samples according to resistance to compression, and, in judging the wool, associated the specific gravity with the resistance to compression. The values given in the table provide no ground for assuming such an association. Furthermore, the variation in specific gravity among wools is so small that it seems unlikely that it can be estimated visually or tactually, unless it is correlated with some more readily estimated wool characteristic. The term in its application should, therefore be eliminated from wool practice as it can only lead to confusion, and the attribute confined to experimental work.

6. HARSHNESS.

In practical wool judgment a number of terms are employed to denote characteristics which through experience have been found to be important, but whose magnitude is estimated subjectively. It is one of the aims of research to determine the factors which are involved in such properties. As an example consider the property known as "handle". Wools are often described as having "kind, good, soft, bad or harsh handle". It would appear that "handle" generally refers to the attributes of harshness or softness, and great importance is attached to these properties. (Hawkesworth, 1920; Cowley, 1928).

Harshness is often associated with the crimping. Heyne (1924) states that softness of handle is not associated with fineness of fibre but requires a pliable fibre with a smooth surface. He further considers that overcrimping

is often associated with hardness and brittleness. Rose (1933) states that "softness of handle in Merino wool is admittedly closely associated with regular crimp formation, but the correlation is by no means absolute". Hawkesworth (1920) and Cowley (1928) associates softness with pliability or flexibility.

In an investigation into the harshness of four yarns, Larose (1934) found that the order of harshness corresponded to the order of resistance to compression, there being no difference between the yarns as regards fibre thickness or number of scales per mm.

In order to study the factors determining harshness, a series of twelve samples was selected for resistance to compression as determined by the dynamic method, and submitted to nine sheep and wool experts. The samples represented different types of merino wool from different sources, and included one cross-bred sample. They had all been washed identically in benzene and water, and had been teased out into as loose a mass as possible, in order to remove all vestiges of a staple form.

The observers were requested to place the samples in order of (1) resistance to compression, and (2) harshness. The placings were compared with the order of resistance to compression as determined by the dynamic method, by calculating Spearman's rank correlation coefficient. The harshness placing was next compared with the order of fibre thickness. The correlation coefficients are given in Table 32.

TABLE 32.

The placings in order of resistance to compression and harshness compared with the measured orders of resistance to compression and fibre thickness, by means of Spearman's rank correlation coefficient.

Subjective Placing of --	Determined Order of—	OBSERVERS.								
		1	2	3	4	5	6	7	8	9
Resistance to compression	Resistance to compression	0.94	0.87	0.60	0.46	0.46	0.39	0.35	0.21	0.18
Harshness.....	Resistance to compression	0	0.13	0.36	0.22	0.58	0.85	0.22	0.13	0.06
Harshness.....	Fibre thickness	0.81	0.96	0.89	0.98	0.29	0.36	0.73	0.94	0.90

A feature of the results was the diversity of opinion among the observers. Observer (1) placed the samples in almost exactly the correct order of resistance to compression, while (2) was also close. Other observers apparently placed a different interpretation on what constituted resistance to compression. As regards harshness, it is evident that all the observers, except (6) paid little attention to resistance to compression when judging the harshness.

Although the samples had not been selected for fibre thickness and did not represent a well-graded series in respect of this characteristic, it is significant that most of the observers gave an order for the harshness in remarkable agreement with the order of fibre thickness. Bearing in mind that the wool had been washed and teased into as loose a mass as possible, so that the

crimping of the staple could not have influenced the observers, it was concluded that fibre thickness was one factor which had influenced the estimation of the harshness of the sample.

This result appeared important enough to justify further investigation. Three of the observers accordingly selected a series of fourteen samples in the grease, particular attention being paid to fine-fibred samples which were harsh, and coarse-fibred samples which were soft. In Table 33 is recorded the description submitted with the samples, together with the resistance to compression *after cleansing*, the mean fibre thickness, number of crimps per inch, and the percentage yield, calculated from the weights of both greasy and clean sample at 65 per cent. relative humidity and 70° F. temperature.

TABLE 33.

The description of the samples subjectively selected for harshness, with the resistance to compression after cleansing, fibre thickness, number of crimps per inch, and percentage yield.

Sample No.	Description.	Resistance to Compression. (Kg. cm. ² per 5 gm.).	Fibre thickness	Crimps per Inch.	Yield.
			Microns.		Per cent.
1	70's.—Excellent handle.....	8.7×10^3	20.6	18.4	57
2	70's.—Common. Harsh handle.....	13.0×10^3	24.3	17.4	49
3	70's.—Ordinary. Fair handle.....	10.5×10^3	21.9	18.3	42
4	66-70's.—Common. Harsh handle.....	10.2×10^3	28.4	12.6	56
5	66's.—Common. Very harsh handle.....	11.1×10^3	24.5	12.8	43
6	64-66's.—Common. Very harsh handle.....	10.9×10^3	24.6	12.4	35
7	64's.—Common. Harsh handle.....	9.2×10^3	23.1	12.5	51
8	64's.—Common. Fair handle.....	7.9×10^3	22.3	9.2	50
9	64's.—Common. Harsh handle.....	10.8×10^3	25.8	12.2	51
10	60-64's.—Common. Harsh handle.....	8.0×10^3	25.5	7.4	52
11	60's.—Good handle.....	5.1×10^3	22.9	9.3	62
12	60's.—Common. Harsh handle.....	10.1×10^3	25.1	10.4	41
13	58's.—Excellent handle.....	4.8×10^3	25.4	7.9	57
14	58's.—Good handle.....	6.4×10^3	23.9	8.3	48

"REMARKS.—Nos. 2, 4, 5 and 6 are fine wools with bad handle. Nos. 11, 13, and 14 are coarse wools with good handle".

It is evident that some of the alleged fine wools had a considerably greater fibre thickness than was supposed, and the observers must have based their estimation of fineness almost entirely on the crimping. Thus, samples 2, 4, 5 and 6, which were regarded as fine-fibred and harsh, in reality had a coarse fibre, so that the effect of fibre thickness is again apparent. Fibre thickness was not, however, the only factor, as is shown by sample 13, described as having an excellent handle. This sample, though coarse-fibred, had a low resistance to compression, so that a combined effect of fibre thickness and resistance to compression in determining the harshness is suggested.

An analysis of the relative importance of the factors concerned in determining the harshness is rendered difficult by the lack of a criterion for harshness, but an approximate analysis was attempted by assigning an index of harshness from (1) to (5) according to the descriptions of "excellent, good,

fair, harsh and very harsh handle" respectively. In order to reduce the factors concerned to a common basis, each was expressed as a ratio of the mean for the group of fourteen samples, as illustrated in Table 34.

TABLE 34.

The index of harshness assigned to each sample, and the attributes of the samples expressed as a ratio of the mean for the group.

Sample No.	Harshness Index (Arbitrary).	AS RATIO OF MEAN OF GROUP.		
		Resistance to Compression.	Fibre thickness.	Percentage Non-Wool Portion.
1.....	1	0.96	0.85	0.85
2.....	4	1.44	1.01	1.01
3.....	3	1.16	0.91	1.15
4.....	4	1.13	1.18	0.87
5.....	5	1.23	1.01	1.13
6.....	5	1.20	1.02	1.29
7.....	4	1.02	0.96	0.97
8.....	3	0.87	0.92	0.99
9.....	4	1.19	1.07	0.97
10.....	1	0.88	1.06	0.95
11.....	2	0.56	0.95	0.75
12.....	4	1.12	1.04	1.17
13.....	1	0.53	1.05	0.85
14.....	2	0.71	0.99	1.03
Regression coefficient.....		2.38	5.96	3.15

Regarding the index of harshness as a linear function of the three variables, the regression coefficients given at the foot of each column are obtained. According to this method of analysis, i.e., with each factor related to its mean value, the greatest contribution to the harshness was given by the fibre thickness, with a regression coefficient of 5.96. The coefficient for the resistance to compression was less than half this value (2.38), while the percentage of non-wool constituents had a slightly greater effect than the resistance to compression, with a coefficient of 3.15.

The degree to which the method of analysis employed gives the correct order of harshness may be judged from Table 35, where the arbitrary harshness index is compared with that calculated from the equation

$$H = 2.38.a.\bar{a} + 5.96.d.\bar{d} + 3.15.b.\bar{b} - 8.21. \quad (32)$$

where H is the harshness index, a the resistance to compression of the clean wool, d the mean fibre thickness, and b the percentage of non-wool impurities ($=100 - \text{percentage yield}$).

The samples have been arranged according to the calculated harshness index, and an examination of the order of the subjective descriptions and harshness index shows reasonably good agreement. It is doubtful whether better agreement is possible, for besides the fact that the harshness is subjectively estimated, it is not expressible in arithmetical terms, and other factors such as the quality of the grease (viscosity, etc.), and the surface friction have not been taken into account.

TABLE 35.

The arbitrary harshness index compared with that calculated from an equation (32) linear in the three variables. Samples in order of the calculated index.

Sample No.	Description of "Handle".	HARSHNESS INDEX.	
		Arbitrary.	Calculated.
11	Good.....	2	1.2
1	Excellent.....	1	1.8
13	Excellent.....	1	2.0
8	Fair.....	3	2.5
14	Good.....	2	2.6
7	Harsh.....	4	3.0
10	Harsh.....	4	3.2
3	Fair.....	3	3.6
9	Harsh.....	4	4.1
4	Harsh.....	4	4.3
12	Harsh.....	4	4.3
5	Very harsh.....	5	4.3
2	Harsh.....	4	4.4
6	Very harsh.....	5	4.8

It may, however, be inferred that fibre thickness is the main factor which determines the harshness, while resistance to compression is a less important, though definite, factor. In the greasy state of the sample, the non-wool impurities also influence the estimation of harshness, though this factor will be absent in scoured wool.

This conclusion agrees with the finding of Larose (1934), for the four yarns examined by him had the same fibre thickness, and the order of harshness corresponded to the order of resistance to compression.

Another possibility, viz., the surface factor, was next investigated, and use was made of the findings of Mercer and Freney (1940). These investigators immersed wool for one minute in a 7 per cent. solution of potassium hydroxide in ethyl alcohol containing 5 per cent. water and 1 per cent. glycerol, followed by a wash in a 5 per cent. solution of sulphuric acid in alcohol. "The treated wool, which has a very slightly harsher handle than normal yarns, was also found to show an increase in the surface friction of individual fibres as quantitatively measured by a method devised by Speakman".

In the present study the same treatment was given to one top sample and three samples of fleece wool washed in benzene and water, the only difference being that sodium hydroxide was used instead of potassium hydroxide. The treated samples were found to be markedly harsher than the untreated, even to untrained observers.

The resistance to compression of the samples was determined, and also the surface friction by Speakman and Stott's (1931) method as modified by Bosman and van Wyk (1941), with the results shown in Table 36.

TABLE 36.

The resistance to compression and the coefficients of friction of wool treated with sodium hydroxide in alcohol (means of duplicates).

Sample.		Resistance to Compression. (Kg. cm. ² per 5 gm.).	Coefficient of friction of fibres moving in direction of -	
			Root.	Tip.
Top.....	Untreated.....	8.5×10^3	0.279	0.405
	Treated.....	8.2×10^3	0.333	0.453
	Difference.....	0.3×10^3	+ 0.054	+ 0.048
Ram 89.....	Untreated.....	7.1×10^3	0.228	0.322
	Treated.....	6.7×10^3	0.299	0.428
	Difference.....	$- 0.4 \times 10^3$	+ 0.071	+ 0.106
Ewe 73.....	Untreated.....	8.5×10^3	0.197	0.267
	Treated.....	8.3×10^3	0.262	0.368
	Difference.....	$- 0.2 \times 10^3$	+ 0.065	+ 0.101
Ram 102.....	Untreated.....	11.2×10^3	0.219	0.332
	Treated.....	10.3×10^3	0.291	0.437
	Difference.....	$- 0.9 \times 10^3$	+ 0.072	+ 0.105

If anything, the resistance to compression had been reduced by the treatment, and could not have been responsible for an increase in the harshness. There was, however, an increase of about 30 per cent. in the coefficient of friction in both directions, and it may be concluded that the increase in harshness was due to the increase in the surface friction.

The increase in the coefficients of friction was remarkably similar for the three benzene-scoured wools, but was smaller in the case of the top sample, presumably owing to the fact that the top sample was the only one which had been soap scoured and combed, either or both of these treatments being responsible for the higher initial coefficients of friction.

In connection with the effect of fibre thickness it is to be noted that, theoretically at least, the resistance to compression of a mass of fibres depends on Young's modulus (by bending) but is independent of the fibre diameter, while the resistance to bending of single fibres depends on both Young's modulus and the fourth power of the diameter. The effect of fibre thickness on the harshness as actually estimated therefore shows that the pliability of single fibres is involved, probably those projecting from the surface and those forming the surface of the fibre mass. A low resistance to compression, in the case of a relatively coarse-fibred sample which is nevertheless soft, indicates a low value of Young's modulus which counteracts the effect of the fibre thickness on the resistance offered by individual fibres to bending.

From the foregoing it must be inferred that in the estimation of harshness and softness a sample is not grasped and compressed firmly, a conclusion which supports the principle adopted by the Eggerts (1925) of regarding the "latent" pressure of the wool at zero applied pressure as an index of the softness, although it has been pointed out that the measurement of this quantity is subject to considerable experimental error.

The effect of the surface friction is obvious. The softness of wool as compared to that of other textiles can hardly be referred to the surface friction, and must be attributed mainly to the pliability of the fibres and the greater volume occupied by a wool sample on account of the crimping. On the other hand the manufacturer's efforts to produce softness in a fabric may be directed towards lowering the surface friction.

7. LIME-SULPHUR DIPPING.*

While the effect of chemical or mechanical treatments on the resistance to compression of the wool did not ordinarily fall within the scope of the present study, the procedure of dipping is carried out prior to shearing and must, therefore, be regarded as a factor in production. Woolmen often regard dipped wool as having undergone changes as a result of the dipping, and buyers are inclined to discriminate against dipped wool. No critical examination of dipped wool as regards its compressional characteristics has been made, so that this aspect has been included.

Possible effects of the dipping are also of importance from the experimental point of view, for the present study included wool from different sources.

The following is an account of a determination of the effect of lime-sulphur dipping on the resistance to compression of the wool. The material comprised 300 gm. samples taken from each of seven fleeces representing different Merino wool types. Staples were drawn from each sample and placed in three lots in succession, so that three similar samples of 100 gm. each were obtained. The first sample was dipped in a 45-gallon solution which had been in use for two and a half years and had been brought up to strength, while the second sample was dipped in a similar quantity of freshly prepared solution. Both solutions contained 1.5 per cent. of polysulphide sulphur.

The samples were each immersed for two minutes and were continually squeezed and agitated so as to ensure thorough contact with the liquid. The third sample was kept as control. After eight days the treatment was repeated so as to conform to practice.

Since dipping usually takes place within three months after shearing, three-quarters of the wool grown during the year is not subjected to the dip, and the procedure followed in the present study must have exaggerated the influence of the dip to a degree not ordinarily met with in practice.

After four months the resistance to compression was determined subsequent to the customary sampling and cleansing, with the results shown in Table 37.

There is no significant difference between the control sample and that dipped in the used dip ($t=1.19$), or between the control sample and that dipped in the fresh dip ($t=0.87$). It must be concluded that the dipping of wool in lime-sulphur dips, as carried out in practice, has no effect on the compressibility of the wool. The wool only was treated, and the possibility of an effect on the animal was, therefore, not taken into account. Any detrimental effect on the animal would be reflected in the properties of the wool.

In collaboration with Mr. P. M. Bekker, of the Section Chemical Pathology.

TABLE 37.

The resistance to compression, fibre thickness, and number of crimps per inch of seven samples dipped in lime-sulphur dip.

Fleece.		Used Dip.	Fresh Dip.	Control.
1	Resistance to compression (Kg. cm. ² per 5 gm.).	7.5 \times 10 ³	7.6 \times 10 ³	7.2 \times 10 ³
	Fibre thickness (microns).....	19.3	18.4	18.9
	Crimps per inch.....	13.4	13.6	13.1
2	Resistance to compression (Kg. cm. ² per 5 gm.).	8.9 \times 10 ³	8.7 \times 10 ³	8.6 \times 10 ³
	Fibre thickness (microns).....	21.6	22.7	22.4
	Crimps per inch.....	12.5	13.0	13.0
3	Resistance to compression (Kg. cm. ² per 5 gm.).	9.1 \times 10 ³	8.8 \times 10 ³	9.3 \times 10 ³
	Fibre thickness (microns).....	28.0	27.4	28.2
	Crimps per inch.....	9.9	10.3	10.1
4	Resistance to compression (Kg. cm. ² per 5 gm.).	9.9 \times 10 ³	9.1 \times 10 ³	8.7 \times 10 ³
	Fibre thickness (microns).....	21.6	21.6	21.8
	Crimps per inch.....	15.7	15.4	16.7
5	Resistance to compression (Kg. cm. ² per 5 gm.).	10.2 \times 10 ³	9.7 \times 10 ³	9.6 \times 10 ³
	Fibre thickness (microns).....	20.9	19.7	19.5
	Crimps per inch.....	15.2	15.0	14.7
6	Resistance to compression (Kg. cm. ² per 5 gm.).	10.5 \times 10 ³	10.7 \times 10 ³	10.7 \times 10 ³
	Fibre thickness (microns).....	21.3	20.9	20.7
	Crimps per inch.....	17.3	17.5	18.1
7	Resistance to compression (Kg. cm. ² per 5 gm.).	11.3 \times 10 ³	11.8 \times 10 ³	11.6 \times 10 ³
	Fibre thickness (microns).....	24.3	24.8	23.3
	Crimps per inch.....	14.9	15.1	14.7
Mean	Resistance to compression (Kg. cm. ² per 5 gm.).	9.6 \times 10 ³	9.5 \times 10 ³	9.4 \times 10 ³
	Fibre thickness (microns).....	22.4	22.2	22.1
	Crimps per inch.....	14.1	14.3	14.3

8. COMPRESSIBILITY OF THE FLEECE IN RELATION TO THE ANIMAL.

(a) *Variation within the fleece.*

The variation of wool attributes within the fleece is important in two respects. In the first place, both breeder and manufacturer attach great importance to uniformity in the fleece, a point which has been stressed by various authors (Hawkesworth, 1920; Heyne, 1924; Barker, 1931; Rose, 1933; Bosman, 1937, *et alia*). In this connection Frölich, Spöttel and Tänzer (1929) state: "For the breeder as well as for the manufacturer, the uniformity of the wool is of the greatest importance. The greater the uniformity of the fleece, the greater is its value in a breeding sense and also as a commodity".

In the second place, the variation plays an important part in experimental work. Studies on the wool characteristics have often been confined to measurements on small samples taken from the live animal, usually from the shoulder, side, belly and britch. Such a procedure can be regarded as justifiable in cases where samples are taken at intervals from the same sheep, and

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the refinement is often employed of tattooing a small area on the skin in order to ensure that successive samples are taken from the same region of the animal. In the case of studies where the differences between sheep are of greater importance, as in genetical studies, the practice of selecting a few samples is unreliable unless the degree and nature of the variability over the fleece are known

In a study of the variation in tensile strength over the fleece (van Wyk, 1941), it was found that the belly sample differed considerably from the rest of the fleece as regards tensile strength. It was further concluded that, should it be necessary to confine tensile strength studies to small samples, a sample from the shoulder region would be the most suitable.

The present study gives the result of compressibility determinations on the same set of samples. These were obtained from eight four-year-old Merino sheep, selected, so as to include different types, from a group which had been reared in a small, bare paddock and fed on an optimum ration for growth and production from the time of weaning. A brief description of each sheep is given in Table 38, the body weights being those obtained immediately after shearing, two months after the samples had been taken.

TABLE 38.

The sheep used for determining the variation over the fleece.

Sheep No.	Sex.	Body Weight. (Kg.).	DESCRIPTION. (By practical methods of judgment).
1	Ewe.....	49	Exceptionally plainbodied. Measurement showed the variation in fibre fineness over the body of the sheep to be exceptionally small. The wool had a soft handle.
2	Ewe.....	45	Extremely wrinkly. The wool had an excellent crimp definition, but results of measurements showed a high variability in fibre fineness.
3	Ram.....	67	Plainbodied. The wool had a shallow type of crimping.
4	Ewe.....	74	Exceptionally large and plainbodied. The ewe was described as a good flock type.
5	Ewe.....	53	Plainbodied. The fleece was extremely hairy, crimping was almost entirely absent, and the wool felt harsh.
6	Ram.....	57	Extremely wrinkly. The wool had a well-defined crimp and was rather short. Measurement showed considerable variation in fibre fineness over the body of the sheep.
7	Ram.....	84	Plainbodied. The wool was long and loose.
8	Ram.....	63	Plainbodied. An extremely hairy fleece, with crimping almost absent. The wool was harsh to the touch.

The eight sheep included widely different types, and might be expected to show extreme values in compressibility.

Samples of approximately 100 gm. weight each were taken from the shoulder, back, side, neck, thigh and belly regions, and the resistance to compression, mean fibre thickness and number of crimps per inch were determined for each sample. The results are given in Table 39.

TABLE 39.

The resistance to compression (in Kg. cm.² per 5 gm.), fibre thickness and number of crimps per inch at different regions of the sheep.

Sheep No.		Shoulder.	Back.	Side.	Neck.	Thigh.	Belly.	Mean per Sheep.
1	Resistance to compression.....	6.1	6.8	7.8	6.5	7.7	7.2	7.0×10^3
	Fibre thickness.....	18.5	17.6	18.3	18.9	18.8	20.6	18.8μ
	Crimps per inch.....	12.7	15.6	15.8	13.7	14.8	12.1	14.1
2	Resistance to compression.....	10.6	10.8	12.0	10.3	11.6	10.4	11.0×10^3
	Fibre thickness.....	23.9	23.8	23.6	25.5	23.7	23.7	24.0μ
	Crimps per inch.....	13.0	15.0	13.6	12.5	13.8	12.2	13.4
3	Resistance to compression.....	7.4	9.1	8.6	9.1	7.4	8.4	8.3×10^3
	Fibre thickness.....	25.1	26.7	25.7	26.8	25.0	26.9	26.0μ
	Crimps per inch.....	10.3	10.0	11.0	10.8	9.9	9.1	10.2
4	Resistance to compression.....	8.7	9.7	11.2	11.8	9.0	9.7	10.0×10^3
	Fibre thickness.....	23.1	22.5	24.3	24.3	25.3	23.9	23.9μ
	Crimps per inch.....	11.0	13.2	13.0	12.6	10.3	11.0	11.9
5	Resistance to compression.....	6.8	7.8	7.1	7.9	6.7	8.7	7.5×10^3
	Fibre thickness.....	26.8	26.6	27.2	28.5	27.9	27.9	27.5μ
	Crimps per inch.....	8.3	9.5	8.5	9.5	7.7	8.9	8.7
6	Resistance to compression.....	11.8	10.9	11.8	12.3	12.0	11.8	11.8×10^3
	Fibre thickness.....	24.8	21.8	26.0	26.7	28.2	27.5	25.8μ
	Crimps per inch.....	12.9	12.8	11.3	11.1	10.7	11.4	11.7
7	Resistance to compression.....	8.8	9.6	9.6	12.5	7.2	9.0	9.5×10^3
	Fibre thickness.....	23.2	21.7	23.4	25.4	23.8	22.4	23.3μ
	Crimps per inch.....	11.5	12.5	12.0	11.9	9.8	10.5	11.4
8	Resistance to compression.....	9.9	9.2	9.9	11.4	10.3	9.6	10.1×10^3
	Fibre thickness.....	25.9	25.8	26.2	27.5	26.6	24.6	26.1μ
	Crimps per inch.....	13.5	11.4	11.9	11.8	10.5	11.7	11.8
Mean per region	Resistance to compression.....	8.9	9.2	9.8	10.2	9.0	9.4	$\times 10^3$
	Fibre thickness.....	23.9	23.3	24.3	25.5	24.9	24.7	μ
	Crimps per inch.....	11.7	12.5	12.1	11.7	10.9	10.9	

An analysis of variance of the resistance to compression, both before and after adjustment for the effect of fibre thickness and crimping, is given in Table 40.

The value of 0.557 for z before adjustment is significant at the 5 per probability level, showing that a significant difference exists between the values obtained on different regions of a sheep. After adjustment the value of z becomes 0.304, which is insignificant. The difference may, therefore, be mainly, though not solely, associated with differences in fibre thickness and crimping. The error variance, representing the inter-action between sheep and regions, differs highly significantly from the variance between duplicate determinations (standard deviation = 0.37×10^3) with a z value of 0.843, showing that the order of the variation is not the same for the different sheep.

TABLE 40.

Analysis of variance of the resistance to compression, before and after adjustment for fibre thickness and crimping.

Variance.	BEFORE ADJUSTMENT.			AFTER ADJUSTMENT.		
	D.F.	Standard Deviation. (Kg. cm. ² per 5 gm.).	<i>z</i>	D.F.	Standard Deviation. (Kg. cm. ² per 5 gm.).	<i>z</i>
Between sheep.....	7	4.061×10^3	0.557	7	2.886×10^3	0.304
Between regions.....	5	1.501×10^3		5	0.914×10^3	
Error.....	35	0.860×10^3		33	0.674×10^3	

On the average, the shoulder and thigh wool offered the lowest resistance to compression, while the neck wool gave the highest values. The striking difference in tensile strength between the belly wool and the samples from other regions, found previously, was not reflected in the resistance to compression, so that the factors which had reduced the tensile strength had not affected the resistance to compression.

The difference between each region and the mean of the six regions is summarised in Table 41.

TABLE 41.

Difference in resistance to compression between each region and the mean of the six regions.

Region.	Mean Difference. (Kg. cm. ² per 5 gm.)	Standard Deviation of Differences. (Kg. cm. ² per 5 gm.)
Shoulder.....	-0.62×10^3	0.447×10^3
Back.....	-0.15×10^3	0.557×10^3
Side.....	$+0.37 \times 10^3$	0.557×10^3
Neck.....	$+0.84 \times 10^3$	1.218×10^3
Thigh.....	-0.40×10^3	1.024×10^3
Belly.....	-0.04×10^3	0.570×10^3

In the case of tensile strength, the standard deviation of the differences of the shoulder sample was found to be so much lower than that of the other regions that it was concluded that the shoulder sample should be employed when it was necessary to confine tensile strength studies to small samples taken from a single region of a sheep. In the case of resistance to compression, the standard deviation of the differences of the shoulder sample is lowest, but not to an extent that would warrant so definite a conclusion, but it may nevertheless be inferred that the shoulder sample, while giving too

low a value for the fleece, will give a value which most consistently represents the value for the fleece on a comparative basis. The use of a shoulder sample for estimating also the fineness of a fleece seems to be suggested by the work of Duerden and Bell (1931).

While the tensile strength of the belly samples was consistently lower than that of the other regions, the resistance to compression of the belly samples was on the average the same as the mean of the six samples. It is also interesting to observe that the neck wool, which on the average gave the highest resistance to compression, also gave the greatest variation in the difference from the mean of the six regions.

Regarding the question of sampling in experimental work, it should be noted that the variation over the fleece has probably been underestimated on account of the relatively large samples taken (100 gm.). In the case of the two wrinkly sheep 2 and 6, for example, the variation in fibre thickness shown in Table 39 is small compared to the variation obtained when the fleeces of these sheep were employed in a fineness sampling experiment. In the latter experiment, single staples were taken as the sampling units, and the difference in fibre thickness between adjacent staples growing on and between skinfolds was so large that a representative sample for the fleece could not be obtained with even 160 samples taken at random from the shorn fleece after zoning. It must be concluded that the variation between regions covered by 100 gm. samples is considerably lower than the variation between the staples composing such a region. While a shoulder sample has been suggested when the taking of several samples is impracticable, it is further recommended that the sample should be as large as possible. If too large, such a sample can be reduced to a smaller one comprising staples taken at random or after zoning, or merely by taking strands from each staple. Such a sub-sample will more satisfactorily represent the fleece than one of the same size taken from a small region. It is clear that this suggestion does not apply when successive samples are taken from the same region of a sheep to determine the effect of various treatments, but to cases where different sheep are compared.

Where possible, however, the author favours the use of a representative sample from the fleece. This method assumes special importance in relation to a system of fleece analysis and recording for breeders. The breeder is relieved of the responsibility of sampling if he submits the entire fleece. The representative sample taken in the laboratory is employed for determining all the fleece attributes, and it is suggested that for this purpose the belly wool should be excluded, since it has a consistently lower tensile strength than the rest of the fleece. It is not thereby suggested that the belly wool should be ignored in breeding practice, and if desired the belly wool could be sampled and analysed separately.

In the present study, representative samples were taken from fleeces and lots. It is to be noted, however, that a representative sample may consist of staples differing in respect of crimping, fibre thickness and compressibility. Determinations on such a sample, and on any blend of samples, when based on equal weights of wool, cannot be considered valid unless the different constituents contribute to the result according to their respective weights.

The point was investigated by blending in different proportions two widely differing samples, whose relevant characteristics are given in Table 42.

TABLE 42.

The resistance to compression, fibre thickness and number of crimps per inch of two samples A and B in a blend.

	A.	B.
Resistance to compression (Kg. cm. ² per 5 gm.).	14.9×10^3	5.1×10^3
Fibre thickness.....	22.9μ	29.3μ
Number of crimps per inch.....	18.8	6.1

The table shows that the two samples represented extremes as regards resistance to compression, and moreover differed widely in respect of both fibre thickness and crimping.

It was necessary to blend the samples so as to produce intimate contact between the fibres of the different samples, and the method adopted was as follows. The samples were first washed in warm benzene without disturbing the staple form, after which representative samples were weighed out, one gm. from A and four gm. from B. Small tufts were taken at a time from each sample, and these were blended so as to produce contact between fibres from the two samples, the parallelism of the fibres aiding the procedure. Other proportions were made up in a similar manner, after which the whole process was repeated in order to obtain duplicate samples. The final composite samples were then subjected to the usual cleansing process, and were teased out so as to destroy the parallelism of the fibres prior to compression.

The resistance to compression of each of the blends is given in Table 43, and also the weighted value as estimated from the values of the original samples.

TABLE 43.

The resistance to compression of various blends made up of two samples A and B whose characteristics are given in Table 42.

PERCENTAGE BY WEIGHT.		RESISTANCE TO COMPRESSION.	
A.	B.	Determined Value. (Kg. cm. ² per 5 gm.)	Weighted Estimated Value. (Kg. cm. ² per 5 gm.)
0	100	5.1×10^3	—
20	80	7.0×10^3	7.1×10^3
40	60	9.2×10^3	9.0×10^3
60	40	11.0×10^3	11.0×10^3
80	20	12.9×10^3	12.9×10^3
100	0	14.9×10^3	—

The determined value is in excellent agreement with the weighted estimate, the agreement being illustrated in Figure 14.

It may be concluded, therefore, that when samples are compared on the basis of equal weights, each constituent of a blend contributes to the resistance to compression by an amount which is in proportion to its weight.

This is not necessarily true for the determination of other properties, as the following two examples illustrate. In the method of cutting fibres into fragments for fibre thickness determinations, such as has been employed in the present study, the different constituents contribute to the measured value by an amount in proportion to their respective lengths, assuming the fragments of the different constituents to have been cut to the same length. In a 1:1 blend by weight of the above samples A and B, the total length of the finer sample exceeds that of the coarser sample in the ratio 1.6:1. A second example occurs in the shrinkage of a cloth on milling, for Speakman and Stott (1931) record that the shrinkage of a cloth consisting of a blend of Merino and Southdown wool is not in proportion to the respective amounts of the two types of wool in the blend.

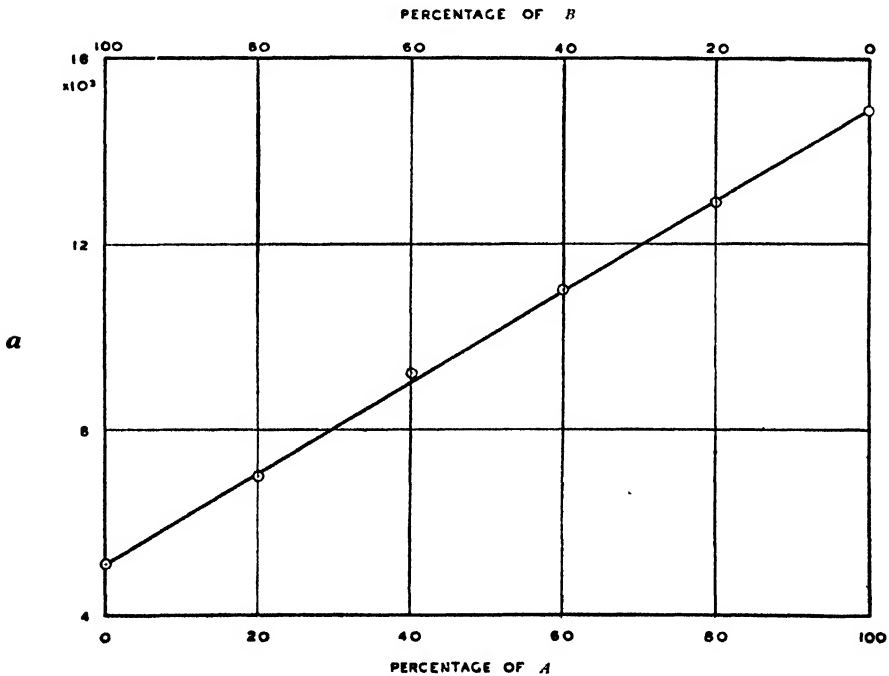


FIGURE 14.—The resistance to compression a of various blends of two samples A and B.

In the case of resistance to compression, the procedure of taking representative samples from a fleece or lot is entirely justifiable, when samples are compared on the basis of equal weights.

(b) Clean Yield of Fleece.

Approximately half of the fleece as shorn from the sheep consists of wool, the remainder being made up of grease, suint, and extraneous impurities such as sand and vegetable matter, together with the water adsorbed mainly by the wool and suint. Besides the type of wool, the percentage of clean wool present determines the monetary value of the fleece.

In breeding practice it is not only the economic aspect of the clean yield which is of importance, for the grease has always been regarded as essential as a protective covering for the individual fibres, and for preserving the staple form and compactness of the fleece.

The presence of the non-wool fleece constituents will almost certainly influence the practical estimation of such fleece attributes as density and compactness, as also the compressional characteristics of the fleece. In view of the fact that breeders rely entirely on subjective estimation of these attributes the complicating effect of the non-wool fleece constituents may be expected to exercise a considerable influence on breeding practice, and for this reason the correlations existing between the amounts of the constituents and the properties of the wool assume importance.

In the present study the clean yield, resistance to compression, fibre thickness and number of crimps per inch of 184 fleeces from various sources were determined. The percentage yield was taken to be the amount of clean dry wool, expressed as a percentage of the "floor" weight of the greasy fleece. While this definition suffers from the defect that the moisture content of the greasy fleece is unknown, it is the one commonly adopted in practice, and the one on which the results became available.

The correlation coefficients between the percentage yield and the fibre characteristics are given in Table 44.

TABLE 44.

The correlation coefficients between resistance to compression, percentage yield of fleece, fibre thickness, and number of crimps per inch of 184 fleeces.

	Resistance to Compression.	Percentage Yield.	Fibre Thickness.	Number of Crimps per Inch
Resistance to compression	—	-0.5674	+0.0835	+0.5020
Percentage yield.....	-0.5674	—	+0.0710	-0.4456
Fibre thickness.....	+0.0835	+0.0710	—	-0.5541
Number of crimps per inch	+0.5020	-0.4456	-0.5541	—

There is a highly significant negative correlation ($r = -0.5674$) between the resistance to compression and the percentage yield of the fleece, showing that in general the high-yielding wools have a low resistance to compression, and vice versa.

After the effects of fibre thickness and crimping have been eliminated, the partial correlation coefficient between resistance to compression and percentage yield becomes -0.3872 . This value is still highly significant, but the reduction shows that part of the total correlation is due to the correlation between resistance to compression and number of crimps per inch ($r = +0.5020$) and the correlation between the number of crimps per inch and the percentage yield ($r = -0.4456$).

In view of the possible influence on breeding, it is of interest to compare the coefficients of correlation between yield and other fibre characteristics with others obtained at the laboratory, or recorded in the literature. A summary is given in Table 45.

TABLE 45.

Summary of correlations between yield and other attributes.

Author.	ATTRIBUTE.				No. of Observations.	Remarks
	Fibre Thickness.	Crimps per Inch.	Fibre Length.	Staple Length.		
Volkman (1927)....	0.59	—	0.35	—	90	"Mollwitz" stud.
	0.47	—	0.45	—	122	"Tscheschnitz" stud.
	0.71	—	—	—	23	"Mollwitz" stud.
	0.66	—	0.64	—	56	"Mollwitz" stud.
Baumgart (1929)....	0.57	—	0.45	—	52	5 studs (ewes).
	0.51	—	0.39	—	60	5 studs (ewes).
Bosman (1937).....	0.08	—	0.66	—	30	Stud ewes.
	0.03	—	—	0.03	16	Stud rams.
This Study.....	0.07	-0.45	—	—	184	Various sources (Table 44).
	-0.01	-0.19	—	—	14	Harshness samples (Table 33).
	—	-0.28	—	0.01	101	Various sources (unpublished).

In contrast to the results of Volkman (1927) and Baumgart (1929) who found such high correlations between yield and fibre thickness and between yield and fibre length that they investigated the possibility of estimating the yield from the two fibre attributes, there appears to be no correlation between yield and fibre thickness among South African wools, but a correlation between yield and number of crimps per inch. It is also interesting to observe that the yield seems to be correlated with the straight fibre length, but not with the staple length.

(c) Sex of sheep.

In the classing and selection of rams, prominence is given to a property known as "substance", which, in part at least, is determined by the resistance to compression of the wool, and there is a widespread impression that wool from rams has more "substance" than wool from ewes. The practical estimation of "substance" is influenced by such factors as the quantity and quality of the grease, but possible differences in the compressibility of the clean wool were investigated.

For a comparison it is necessary that the two groups, rams and ewes, shall have been subjected to the same factors likely to influence the wool. Since the main factors are breeding and nutrition, fleeces were compared from the same flock or stud where the sexes had received identical treatment. In addition, flocks were taken in which selection for resistance to compression had not been practised.

First group.

The first group considered was that employed in a genetic experiment which had been commenced with 30 stud ewes, all the progeny of one sire. The same sire was employed in the experiment, and later one of his progeny. No culling had been practised, and the rams and ewes, except for being separated in adjacent camps, were given identical treatment.

Ten fleeces from rams and ten from ewes were selected at random after one shearing. On investigation it appeared that the ages of the rams varied from two and a half years to three and a half years with an average of two and three-quarter years, while those of the ewes varied between the same limits with an average of three years. It has been found [see Section 8 (*d*)] that the resistance to compression of fleeces grown by other sheep did not change from the second to the third years, and the two groups may thus be regarded as comparable in respect of age.

Representative samples were taken from the fleeces after removal of locks and bellies, and the resistance to compression, fibre thickness, and number of crimps per inch were determined. The results are given in Table 46, in the order of decreasing resistance to compression.

TABLE 46.

The resistance to compression, fibre thickness, and number of crimps per inch of wool from rams and ewes. (First group.)

RAMS.				EWES.			
Ram No.	Fibre Thickness (Microns).	Crimps per Inch.	Resistance to Compression (Kg. cm. ² per 5 gm.).	Ewe No.	Fibre Thickness (Microns).	Crimps per Inch.	Resistance to Compression (Kg. cm. ² per 5 gm.).
1	21.5	15.8	10.8×10^3	1	22.9	18.8	14.6×10^3
2	23.7	15.6	10.5×10^3	2	22.5	14.7	13.5×10^3
3	22.2	14.9	10.1×10^3	3	24.1	14.7	10.2×10^3
4	25.9	11.5	9.7×10^3	4	24.5	13.5	9.3×10^3
5	21.6	13.6	9.1×10^3	5	22.5	11.6	8.9×10^3
6	22.7	14.4	8.5×10^3	6	23.6	12.0	8.8×10^3
7	23.1	14.2	8.4×10^3	7	21.4	13.9	8.4×10^3
8	21.5	13.5	8.2×10^3	8	21.0	12.1	7.3×10^3
9	21.3	13.0	7.4×10^3	9	18.6	14.2	7.0×10^3
10	19.6	10.0	5.1×10^3	10	19.8	13.9	7.0×10^3
Mean.....	22.3	13.7	8.8×10^3	Mean.....	22.1	13.9	9.5×10^3

The slightly higher value of the ewes' wool did not differ significantly from that of the rams' wool ($t=0.72$). The effect of fineness and crimping was determined by comparing the variance in the resistance to compression with that obtained after adjustment, assuming a linear relation and calculating the partial regression coefficients from the variance within the groups. The results are given in Table 47.

TABLE 47.

Analysis of variance of resistance to compression, before and after adjustment for the effects of fibre thickness and crimping (First Group.)

Variance.	BEFORE ADJUSTMENT.		AFTER ADJUSTMENT.	
	D.F.	Standard Deviation. (Kg. cm. ² per 5 gm.).	D.F.	Standard Deviation. (Kg. cm. ² per 5 gm.).
Between sexes.....	1	1.610×10^3	1	1.382×10^3
Within sexes.....	18	2.207×10^3	16	1.176×10^3

The difference in the resistance to compression between the sexes was still insignificant after adjustment for the effects of fibre thickness and crimping ($z = 0.161$). The insignificance of the differences between the sexes could not therefore be due to a chance distribution of fibre thickness and crimping between the two groups.

Second group.

The second group of fleeces was derived from a stud in which the breeder had consistently bred for one type of sheep for at least ten years. The fleeces of fifty stud ewes considered to be typical of the stud were submitted for testing, and it was found that the fleeces were remarkably similar as regards mean fibre thickness, for the coefficient of variability of mean fibre thickness was only 4 per cent. Moreover, the ewes were all (according to teeth) of the same age, viz., 4-tooth.

The breeder also submitted 36 fleeces from his top sires, varying in age from 2-tooth to 6-tooth. Again a remarkable uniformity in mean fibre thickness was revealed, the coefficient of variability of mean fibre thickness being 6 per cent. The mean fibre thickness of the ram fleeces was, moreover, found to be identical to that of the ewe fleeces.

The breeder expressly stated that he did not select for "substance", so that it was unlikely that any bias in the matter of resistance to compression could be present. The sheep had all been run on Karroo pasture, and no difference had been made in the treatment of the rams and of the ewes.

Ten fleeces were taken at random from each group, and representative samples drawn from each fleece as before. The results of the measurements are given in Table 48, in the order of decreasing resistance to compression.

Again there was no difference between the sexes as regards the resistance to compression of the wool ($t = 0.10$). An analysis of the variance of the resistance to compression is given in Table 49.

Even after adjustment for fibre thickness and crimping, the whole of the difference between the sexes could be accounted for by the variation within the groups.

TABLE 48.

The resistance to compression, fibre thickness and number of crimps per inch of wool from rams and ewes. (Second group.)

RAMS.				EWES.			
Ram No.	Fibre Thickness. (Microns).	Crimps per Inch.	Resistance to Compression. (Kg. cm. ² per 5 gm.).	Ewe No.	Fibre Thickness. (Microns).	Crimps per Inch.	Resistance to Compression. (Kg. cm. ² per 5 gm.).
1	22.4	10.5	8.3×10^3	1	21.5	12.0	8.6×10^3
2	22.6	11.3	8.2×10^3	2	22.2	12.0	8.1×10^3
3	21.8	10.2	8.0×10^3	3	21.9	12.2	7.7×10^3
4	22.1	9.9	7.8×10^3	4	22.3	11.7	7.5×10^3
5	21.5	12.2	7.7×10^3	5	22.6	11.2	7.5×10^3
6	21.2	11.2	6.9×10^3	6	22.1	12.6	7.2×10^3
7	21.6	10.4	6.9×10^3	7	21.2	9.8	6.9×10^3
8	21.7	7.5	6.8×10^3	8	21.6	12.2	6.6×10^3
9	21.5	11.7	6.6×10^3	9	21.8	10.0	6.2×10^3
10	20.2	8.7	4.9×10^3	10	20.2	10.6	6.2×10^3
Mean.....	21.7	10.4	7.2×10^3	Mean.....	21.7	11.4	7.3×10^3

TABLE 49.

Analysis of variance of resistance to compression, before and after adjustment for the effects of fibre thickness and crimping. (Second group.)

Variance.	BEFORE ADJUSTMENT.		AFTER ADJUSTMENT.	
	D.F.	Standard Deviation. (Kg. cm. ² per 5 gm.).	D.F.	Standard Deviation. (Kg. cm. ² per 5 gm.).
Between sexes.....	1	0.089×10^3	1	0.551×10^3
Within sexes.....	18	0.915×10^3	16	0.631×10^3

In the case of two groups of sheep, therefore, on the average no difference in resistance to compression between the wool from rams and that from ewes has been found, and the results suggest that where systematic differences are encountered, the reason must be sought for in other factors than merely those of sex.

(d) *Age of sheep.*

The changes which take place in the fleece characteristics with the age of the sheep are of importance to the breeder and wool producer. The knowledge of such changes enables the sheep breeder to predict the fleece characteristics of the adult sheep from those of the young sheep, a point which is often useful in assessing the degree to which Merino sires transmit the desired fleece characteristics.

Moreover, when Merino fleeces are judged, allowances have sometimes to be made for differences in the ages of the sheep concerned.

Little research work is on record where the influence of age has been directly investigated. Bosman and van Wyk (1941) found a reduction with age in the percentage difference between the coefficients of friction in the two directions of the fibres composing uniformly grown staples. No record of measurements of the influence of age on the resistance to compression is available.

In studies of this nature it is essential that the sheep shall have been kept under uniform and controlled conditions, especially of feed. Eight sheep were accordingly selected from a group which had been kept on an optimum ration for growth and production since the time of weaning, and had been reared together in a small, bare paddock. The selection included different fleece types.

Representative samples were taken from the fleeces each year, and the resistance to compression, fibre thickness and number of crimps per inch were determined. The experiment is still proceeding, but the results of the first four years are given in Table 50.

The extent to which the differences between the years are associated with the difference in fibre thickness and crimping, may be judged from the variance and co-variance analysis of Table 51.

While the variance "between years" differs significantly from the error variance at the 5 per cent. probability level before adjustment ($z=0.624$), it equals the error variance after adjustment ($z=0.009$). It is evident, therefore, that the differences between the years may be directly attributed to the changes in fibre thickness and crimping with age.

Taking averages for the group (Table 50), the resistance to compression showed an increase with age, the difference between the first and fourth years being 1.4×10^3 (Kg. cm.² per 5 gm.), with a t value of 2.57 which is significant at the 5 per cent. probability level. This was accompanied by an increase in the fibre thickness, and a small increase in the number of crimps per inch. With the regression coefficients of resistance to compression on fibre thickness of 730 and on the number of crimps per inch of 1,006, it is evident that the contribution of the change in the fibre thickness of 1.8μ was large compared to the contribution of the number of crimps per inch, which increased by only 0.5.

In the case of sheep Nos. 6 and 7, the resistance to compression tended to diminish with age, while the fibre thickness did not show the increase evident in the other fleeces. In general it may be inferred that on the average the resistance to compression increases with age for the first four years, but exceptions to this rule occur in cases where the fibre thickness does not increase.

It is interesting to note the large reduction in the variance between sheep, showing that a large part of the differences between the sheep was due to differences in fibre thickness and crimping, although the value of z (0.849) after adjustment was still highly significant.

It is evident from the results that the age of the sheep should be taken into account when judging the fleece. The case of the two sheep which failed to show the increase in resistance to compression illustrates the individual variation among sheep with the consequent difficulties encountered in

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predicting the fleece characteristics of the adult sheep from those of the lamb. In flock improvement, where large numbers are ordinarily dealt with, it is convenient to practise selection at as early an age as possible, and exceptions to the general rule do not have such serious consequences as in stud breeding, where each individual sheep plays an extremely important part.

TABLE 50.

The resistance to compression, fibre thickness, and number of crimps per inch for the first four years.

Sheep No.		YEAR OF GROWTH.				Mean per Sheep.
		First.	Second.	Third.	Fourth.	
1	Resistance to compression..	7.2	8.5	8.4	9.3	8.4×10^3 (Kg. cm. ⁷ per 5 gm.). 22.2μ 11.2
	Fibre thickness.....	20.5	22.8	21.9	23.4	
	Crimps per inch.....	11.2	11.2	9.7	12.5	
2	Resistance to compression..	8.6	11.7	11.1	10.9	10.6×10^3 (Kg. cm. ⁷ per 5 gm.). 23.1μ 11.9
	Fibre thickness.....	22.4	23.5	22.9	23.4	
	Crimps per inch.....	11.9	11.4	12.8	11.4	
3	Resistance to compression..	10.3	10.1	10.7	11.6	10.7×10^3 (Kg. cm. ⁷ per 5 gm.). 26.5μ 11.9
	Fibre thickness.....	25.9	27.9	25.5	26.5	
	Crimps per inch.....	9.8	10.4	10.2	11.9	
4	Resistance to compression..	10.0	13.3	13.6	14.6	13.1×10^3 (Kg. cm. ⁷ per 5 gm.). 24.2μ 13.5
	Fibre thickness.....	21.2	25.6	24.9	25.1	
	Crimps per inch.....	13.3	13.4	13.4	13.8	
5	Resistance to compression..	8.4	9.1	9.6	10.5	9.4×10^3 (Kg. cm. ⁷ per 5 gm.). 24.0μ 9.8
	Fibre thickness.....	21.5	24.2	25.8	24.5	
	Crimps per inch.....	9.6	9.9	9.4	10.4	
6	Resistance to compression..	8.1	7.4	6.8	7.4	7.4×10^3 (Kg. cm. ⁷ per 5 gm.). 19.0μ 12.5
	Fibre thickness.....	19.4	18.5	19.1	18.8	
	Crimps per inch.....	12.6	12.2	12.6	12.7	
7	Resistance to compression..	9.9	9.6	7.5	8.8	8.9×10^3 (Kg. cm. ⁷ per 5 gm.). 23.3μ 10.8
	Fibre thickness.....	24.2	23.5	21.5	24.0	
	Crimps per inch.....	11.1	11.1	10.7	10.4	
8	Resistance to compression..	9.2	9.8	11.1	11.2	10.3×10^3 (Kg. cm. ⁷ per 5 gm.). 23.1μ 12.3
	Fibre thickness.....	20.9	22.4	24.3	24.8	
	Crimps per inch.....	11.9	11.7	13.3	12.3	
Yearly mean	Resistance to compression..	9.1	9.9	9.8	10.5×10^3	(Kg. cm. ⁷ per 5 gm.). 23.8μ 11.9
	Fibre thickness.....	22.0	23.6	23.2	23.8	
	Crimps per inch.....	11.4	11.4	11.5	11.9	

TABLE 51.

Analysis of variance of resistance to compression, before and after adjustment for fibre thickness and crimping.

Variance.	BEFORE ADJUSTMENT.			AFTER ADJUSTMENT.		
	D.F.	Standard Deviation. (Kg. cm. ² per 5 gm.).	<i>z</i>	D.F.	Standard Deviation. (Kg. cm. ² per 5 gm.).	<i>z</i>
Between years.....	3	1.698×10^3	0.624	3	0.431×10^3	0.009
Between sheep.....	7	3.475×10^3	1.340	7	0.998×10^3	0.849
Error.....	21	0.910×10^3	—	19	0.427×10^3	—

An interesting feature of the results is the striking reduction in the variation between the years as a result of the adjustment for fibre thickness and crimping. This result is in contrast to the residual variation among different sheep, and even among the regions of a sheep, and suggests that the other factors which influence resistance to compression either remain constant or alter with the fibre thickness and crimping in a manner which is constant for a sheep. Although the results refer to wool grown under uniform conditions, it is reasonable to conclude that a pre-experimental period in sheep experiments will increase the accuracy greatly as far as resistance to compression is concerned. The same conclusion has been reached with regard to other fleece characteristics, for Malan, van Wyk and Botha (1935), found that approximately 60 per cent. of the variation in fleece attributes during one year could be expressed in terms of that of the previous year, and it was concluded that for the same accuracy the number of sheep in an experiment had to be approximately five times as great when no pre-experimental results were available.

(e) *Feed.*

Wool studies in regard to nutrition have been confined to the characteristics of fibre length, fibre thickness, crimping, tensile strength and the total wool production, and marked effects on these characteristics have been recorded.

The only recorded results of compressibility determinations in regard to nutrition are those of Swart and van Rensburg (in course of publication). These authors examined the phenomenon of straight-fibred wools in the south-western districts of the Cape, and found that the crimping could be restored by the addition of certain supplements to the diet of the sheep. In spite of changes in the fibre thickness and crimping, the resistance to compression remained constant in some cases, and altered in others.

In the present study compressibility measurements were made on the wool grown by sheep under controlled feeding conditions.

After a preliminary period of three months on veld hay, the sheep were divided into four groups, designated (1), (2), (3) and (4), and fed on the following rations:—

First period (9 months): (1) Lucerne, (2) Green Feed, (3) Maize, (4) Oats.

Second Period (3 months): Veld Hay (All Groups).

Third Period (12 months): (1) Maize, (2) Oats, (3) Lucerne, (4) Green Feed.

The rations fed during the first and third periods were given *ad lib.* During the second period, when the sheep had access to veld hay only, they became so emaciated that the period could not be prolonged beyond three months. The average body weight dropped from 38 Kg. to 30 Kg.

At the end of the third period, samples were taken from the shoulder region of each sheep, and the staples were cut into three portions corresponding to the three periods. The divisions were clearly visible and the portions were readily separated.

The wool grown during the undernourished period was found to have lost its staple form to such an extent that it was impossible to measure the crimping, and only fibre thickness measurements were made in conjunction with the compressibility tests.

The results showed no difference between the rations fed during the well-fed periods, and for the purpose of the present study, the periods will, therefore, be regarded only as well-fed and under-fed periods. The experimental results are given in Table 52.

The fibre thickness suffered a definite reduction as a result of the underfeeding, but there was no corresponding change in the resistance to compression. In the absence of data on the crimping, the cause of the failure of the plane of nutrition to influence the resistance to compression cannot be established with certainty, but it can be assumed that the number of crimps per inch of individual fibres had increased, although the crimping was not defined in the staple form. The effects of the reduction in fibre thickness and the increase in the number of crimps per inch may, therefore, have counteracted each other, but an influence on the elastic properties must also be considered.

While the criticism may be made that the period of underfeeding was too short, it may be pointed out that the condition of the animals after three months was such that the period could not be extended, and in fact several sheep were lost during this period.

The results apply only to the conditions of the experiment, and cannot be applied to other nutritional conditions, but they illustrate one type of effect which may occur, viz., a reduction in fibre thickness with no corresponding alteration in compressibility.

Besides the results obtained by Swart and van Rensburg, in which inconsistencies occurred as regards the resistance to compression, a few instances to illustrate the complexity of the nutritional effect may be mentioned.

One instance is afforded by a sample submitted by a breeder, who stated that on transferring his sheep from one farm to another, the wool lost "substance". Although the sample was unsuitable for a determination, actual examination suggested a low resistance to compression, which was presumably due to the fact that while the crimping indicated a 60's wool, the fibre thickness corresponded to a 70's quality number. In this case, therefore, the nutritional effect had been greater on the fineness than on the crimping. This is usually the case with "hungerfine" wools, and it is for

this reason that Duerden stated that his standards were not applicable to droughty or impoverished wools. Swart (1936) also found indications that the quantity of feed had a greater effect on the fineness than on the crimping.

TABLE 52.

The resistance to compression and fibre thickness of wool from sheep kept at different planes of nutrition.

Sheep No.		Well-fed.	Under-fed.	Well-fed.
1	Resistance to compression.....	6.6×10^3	6.6×10^3	7.0×10^3
	Fibre thickness.....	17.0μ	16.6μ	(Kg. cm. ⁷ per 5 gm.). 18.1μ
2	Resistance to compression.....	8.3×10^3	8.8×10^3	9.0×10^3
	Fibre thickness.....	20.3μ	16.5μ	(Kg. cm. ⁷ per 5 gm.). 17.8μ
3	Resistance to compression.....	5.9×10^3	6.4×10^3	6.7×10^3
	Fibre thickness.....	19.0μ	18.1μ	(Kg. cm. ⁷ per 5 gm.). 21.1μ
4	Resistance to compression.....	8.1×10^3	8.7×10^3	6.9×10^3
	Fibre thickness.....	19.0μ	17.6μ	(Kg. cm. ⁷ per 5 gm.). 23.5μ
5	Resistance to compression.....	7.1×10^3	7.4×10^3	6.5×10^3
	Fibre thickness.....	22.0μ	17.6μ	(Kg. cm. ⁷ per 5 gm.). 21.2μ
6	Resistance to compression.....	6.8×10^3	6.6×10^3	7.0×10^3
	Fibre thickness.....	16.6μ	15.2μ	(Kg. cm. ⁷ per 5 gm.). 19.4μ
7	Resistance to compression.....	7.9×10^3	6.8×10^3	7.6×10^3
	Fibre thickness.....	23.3μ	18.1μ	(Kg. cm. ⁷ per 5 gm.). 21.5μ
8	Resistance to compression.....	6.7×10^3	8.0×10^3	7.2×10^3
	Fibre thickness.....	18.9μ	15.9μ	(Kg. cm. ⁷ per 5 gm.). 21.1μ
9	Resistance to compression.....	8.4×10^3	7.1×10^3	7.0×10^3
	Fibre thickness.....	19.7μ	16.9μ	(Kg. cm. ⁷ per 5 gm.). 21.7μ
Mean...	Resistance to compression.....	7.3×10^3	7.4×10^3	7.2×10^3
	Fibre thickness.....	19.5μ	16.9μ	(Kg. cm. ⁷ per 5 gm.). 20.6μ

The complexity of the nutritional effect is not, however, confined to the relation between the fineness and the crimping, as was shown by a staple submitted by another breeder. The number of crimps per inch showed a relatively sudden change from 10 to 16 at about the middle of the staple, and the crimping was regular and well-defined throughout both portions. The fibre thickness showed a corresponding reduction from 23μ to 18μ , but the remarkable feature lay in the variability in fibre thickness, for the standard deviation was the same for the two portions, viz., 3.4μ . The result was an increase in the coefficient of variability from 15 per cent. to 19 per cent.

This result is in direct contrast to the findings of Malan, van Wyk and Botha (1935), for the coefficient of variability in the case of 50 sheep remained sensibly constant in spite of relatively large changes in the mean values, and it was concluded that for each sheep there was a proportionate relation between the standard deviation and the mean, determined by genetic factors only. The same conclusion appears to emerge from the results of Swart (1936), for he states: "Quantity of feed, although affecting the fibre diameter, has very little influence on its frequency distribution".

The influence of feed offers a wide field of investigation, as regards not only the plane of nutrition, but also the composition of the ration, the latter phase assuming special importance owing to the deficiencies occurring in certain areas of the Union.

9. THE DISTRIBUTION OF COMPRESSIBILITY.

The range and the distribution of the properties of South African Merino wool can only be determined on representative samples. A truly representative selection of the Union's wool clip is difficult to obtain, since it is necessary that each wool growing area and each type of wool in the area should be taken into account in its correct proportion. Such factors as pasturage and climate vary during a season and from season to season, and any set of observations would consequently apply to a particular period only.

In the present study no systematic attempt was made to procure so representative a series of samples. The samples tested were nevertheless obtained from many different sources, and included stud wools from various breeders, lots submitted by farmers and brokers for testing, prize wools from shows, and wool from experimental sheep in different areas. It is reasonable to assume that a fair representation has been achieved, and the results provide features of general interest.

The frequencies and percentage frequencies of the resistance to compression, a , as defined in Part I of the study, are given in Table 53.

TABLE 53.

The frequencies and percentage frequencies of the resistance to compression of all samples tested.

Resistance to Compression. (Kg. cm. ² per 5 gm.).	Frequency.	Percentage Frequency.
4-5 × 10 ³	3	1.0
5-6 × 10 ³	16	5.2
6-7 × 10 ³	37	11.9
7-8 × 10 ³	65	21.0
8-9 × 10 ³	55	17.7
9-10 × 10 ³	54	17.4
10-11 × 10 ³	47	15.2
11-12 × 10 ³	17	5.5
12-13 × 10 ³	8	2.6
13-14 × 10 ³	5	1.6
14-15 × 10 ³	3	1.0
	310	100.1

Mean..... = 8.7×10^3 (Kg. cm.² per 5 gm.).
 Standard Deviation..... = 1.92×10^3 (Kg. cm.² per 5 gm.).
 Coefficient of Variability..... = 22 per cent.

The distribution is evidently not symmetrical, since it tails off towards the higher values. Testing for normality by calculating g_1 and g_2 (Fisher, 1932) the value of $0.405 \pm 0.139^*$ for g_1 is highly significant at the 1 per cent. probability level. The distribution must, therefore be regarded as being distinctly skew, suggesting that, of the values in the vicinity of the mean, the lower values are more frequent than the higher values, while very high values are relatively more frequent than very low values. The quantity g_2 , a measure of the "flatness" of the curve, has the value of 0.044 ± 0.276 , which is insignificant, so that the distribution cannot be regarded as departing from normality in this respect.

On the other hand, the logarithms of the values may be taken to be normally distributed, for $g_1 = -0.148 \pm 0.139$, and $g_2 = -0.006 \pm 0.276$, both values being insignificant.

The values for resistance to compression range from 4.5×10^3 to 14.6×10^3 (Kg. cm.² per 5 gm.). Among South African Merino wools, therefore, a range occurs of at least 3:1. The mean value is 8.7×10^3 Kg. cm.² per 5 gm., and the standard deviation 1.92×10^3 Kg. cm.² per 5 gm., giving a coefficient of variability of 22 per cent.

The range of values indicates that the manufacturer has at present a considerable choice in the matter of resistance to compression, when selecting a wool to meet his specific requirements.

In the following analysis of the differences between groups, use will be made of the two regression coefficients of resistance to compression on fibre thickness and on number of crimps per inch, as obtained from all the samples tested (equation 30). An adjustment to the mean values of fibre thickness (21.5 μ) and the number of crimps per inch (12.5) will therefore be made by means of the equation

$$a' = a + 357(21.5 - d) + 623(12.5 - n) \dots \dots \dots (33)$$

where a is the resistance to compression as defined, d the fibre thickness in microns, and n the number of crimps per inch.

A study of the effects of environment and pasturage is complicated by variations within a small area as a result of breeding and other factors. A case in point is afforded by the samples employed for determining the differences between rams' wool and ewes' wool, as recorded in Section 8(c). The two groups were bred in adjacent districts, and were run on the same type of pasture (Karoo), so that a difference between them must be attributed almost entirely to breeding.

The means of the groups are recorded in Table 54, and the adjusted values are given in the last column.

There is a significant difference between the two groups as regards resistance to compression ($t=3.6$), which, however, disappears as a result of the adjustment. There is little difference in the mean fibre thickness, so that the major part of the difference between the groups is due to the difference in the number of crimps per inch.

* The standard error is employed throughout.

TABLE 54.

The mean fibre thickness, number of crimps per inch, and resistance to compression of the wool from two groups of sheep in adjoining districts, and the resistance to compression adjusted to the mean value of fibre thickness and crimping.

Group.	Fibre Thickness. (Microns).	Crimps per Inch.	Resistance to Compression. (Kg. cm. ² per 5 gm.).	Adjusted Resistance to Compression. (Kg. cm. ² per 5 gm.).
First.....	22.2	13.8	9.1×10^3	8.2×10^3
Second.....	21.7	10.9	7.2×10^3	8.3×10^3

In the first group the sheep had been employed in a breeding experiment, and no culling had taken place. If this group were to be replaced by a stud where, as is often the case, sheep are among other characteristics selected for the "substance" of their wool, the difference between the groups would probably be greater, since the breeder of the second group expressly stated that he did not breed for "substance".

Karoo and Grassveld wool.

In the marketing of South African wool, brokers and buyers discriminate between Karroo and Grassveld wools, and regard them as two distinct types, each with its own peculiar properties. While the variation within each type is large, an attempt was made to compare the two types by examining samples from a wool show. The wools on show were all prize wools and were authentic representatives of the two types. They consisted of an equal number of each type.

The results of the measurements are given in Table 55, where the means have also been adjusted to correspond to the mean values of fibre thickness and crimping.

The two groups do not differ significantly as regards resistance to compression ($t=0.39$). As a result of the adjustment, the Grassveld wool has a lower resistance to compression than the Karroo wool, although with the small number of observations the difference cannot be regarded as significant ($t=1.60$).

Hence, on the same basis of fibre thickness and crimping, Grassveld wool shows a tendency towards a lower resistance to compression than Karroo wool, but on the average no difference may exist owing to a possibly greater number of crimps per inch of the Grassveld wools.

Various wool growing areas.

A summary of the mean values obtained for various wool growing areas is given in Table 56, in increasing order of resistance to compression.

TABLE 55.

The fibre thickness, number of crimps per inch and resistance to compression of samples from the Karroo and Grassveld areas, obtained at wool show.

KARROO.				GRASSVELD.			
Sample.	Fibre Thickness. (Microns).	Crimps per Inch.	Resistance to Compression. (Kg. cm. ² per 5 gm.).	Sample.	Fibre Thickness. (Microns).	Crimps per Inch.	Resistance to Compression. (Kg. cm. ² per 5 gm.).
1	18.8	14.4	7.6×10^3	1	19.8	14.4	6.9×10^3
2	21.3	12.8	7.8×10^3	2	20.0	15.0	8.2×10^3
3	21.3	14.2	8.8×10^3	3	19.0	16.5	8.4×10^3
4	22.8	12.5	9.1×10^3	4	20.6	16.7	8.8×10^3
5	22.1	12.4	9.3×10^3	5	19.6	15.1	9.1×10^3
6	22.5	15.1	9.3×10^3	6	19.6	15.3	9.4×10^3
7	20.9	16.3	9.6×10^3	7	22.9	14.0	9.7×10^3
8	17.9	17.2	9.7×10^3	8	19.4	15.6	9.8×10^3
9	21.0	12.2	9.8×10^3	9	21.6	14.8	10.1×10^3
10	19.0	16.4	10.9×10^3	10	20.3	17.6	10.2×10^3
11	21.9	14.7	11.6×10^3	11	18.9	18.3	10.8×10^3
Mean.....	20.9	14.4	9.4×10^3	Mean.....	20.2	15.8	9.2×10^3
Adjusted.....			8.5×10^3	Adjusted.....			7.7×10^3

TABLE 56.

The resistance to compression of wool from various wool growing areas.

Area.	No. of Samples.	Fibre Thickness. (Microns)	Crimps per Inch.	RESISTANCE TO COMPRESSION. (Kg. cm. ² per 5 gm.).		
				Range.	Mean.	Adjusted.
Transvaal Grassveld.....	12	20.4	12.5	5.4-10.3	7.4	7.9×10^3
South Western Cape Districts.....	27	20.3	12.5	4.5-10.5	7.8	8.3×10^3
Eastern Province Grassveld.....	11	20.2	15.8	6.9-10.8	9.2	7.7×10^3
Karoo.....	78	21.2	12.9	5.1-14.6	9.2	9.2×10^3
Basutoland.....	22	20.2	14.4	7.7-13.9	9.9	9.3×10^3

While the small number of observations in each group does not permit of general conclusions, certain features of the results may be mentioned.

Transvaal Grassveld.

The lowest average was given by a series of samples grown in the Transvaal Grassveld and obtained at a wool show. The mean was increased by the adjustment for fibre thickness and crimping, but it must be concluded that wool from this area has a lower resistance to compression than Merino wools generally, even taking into account the fibre thickness and crimping.

South-western Districts of the Cape.

Part of the samples tested was obtained from a broker who had specially selected different types, and though the types may have been representative they may not have been present in their correct proportions. Part was derived from a supplementary feeding experiment and the inclusion of these samples was considered to be justified by the lack of a definite difference between the treated and untreated groups, but on the other hand, the samples were grown in one area. The selection of samples cannot, therefore, be regarded as truly representative of the south-western districts, but their low average can hardly be due to chance. The adjustment for fibre thickness and crimping increased the resistance to compression to 8.3×10^3 Kg. cm.² per 5 gm., which is still below the average of 8.7×10^3 Kg. cm.² per 5 gm. The results confirm the conclusion of Swart and van Rensburg, who stated that Western Province wools had a low resistance to compression. The low average may to some extent be attributed to the fineness of fibre, since the number of crimps equals the average. In addition it is to be noted that several of the samples were of the so-called "straight" type, whose fibres were on examination found to have a shallow type of crimping.

Eastern Province Grassveld.

This series of samples was obtained from a wool show, and the results have been considered in a comparison with Karroo wool (Table 55). It was inferred that there was a tendency for Grassveld wools to have a lower resistance to compression than Karroo wools on the same basis of fibre thickness and crimping, but that a possibly greater number of crimps per inch might in practice increase the resistance to compression of the Grassveld wools to equal that of the Karroo wools.

Karoo.

The high average of Karroo wools remained unaltered by the adjustment. It may be concluded that as far as the Union is concerned, Karroo wools offer the greatest resistance to compression.

Basutoland.

The Basutoland samples were the result of a definite attempt, for two years in succession, to obtain as representative a series as possible from the various wool growing areas. The average resistance to compression is exceptionally high, but after adjustment for fibre thickness and crimping, it equals that of Karroo wool. Since the mean fibre thickness is lower than that of Karroo wool, the inordinately high resistance to compression must for the greater part be attributed to the number of crimps per inch.

DISCUSSION (PART II).

Wool is the most important pastoral product of the Union, so that the future of wool is a major issue to the country. Until quite recently wool has always been acknowledged as the supreme clothing material by virtue of its unique properties, but this superiority is now being seriously threatened by competition from artificial fibres.

While world wool production has increased by some 30 per cent. during the last twenty years, that of artificial fibres has increased about fifty-fold. Admittedly a portion of these textiles are not intended to supplant wool,

and are not suitable for this purpose, but a considerable portion consists of a number of types of staple fibre, which if not supplanting wool entirely at present, at least are used in mixtures with wool fibres. It is stated that during the past four years, the production of staple fibre has increased six-fold in two countries alone. Within a short space of time, therefore, the production and consumption of staple fibre have increased enormously.

Although no fibres have yet been produced which possess simultaneously all the desirable properties of wool as clothing material, this fact is hardly a cause for consolation. Recent improvements in all types of artificial fibre, including casein fibre, the production of twist and crimp in the cellulose staple fibre, and the introduction of the truly synthetic fibres such as Nylon, whose properties can be readily varied, show the large measure of success which has already crowned the efforts of the manufacturer of artificial fibres in this direction.

In many respects the producer of artificial fibres has a considerable advantage over the wool grower. Once research has shown a method of improving the artificial product or of reducing production costs, the manufacturer can avail himself of the result almost immediately. The wool producer, on the other hand, can alter his product only by breeding, the results of which become established only after many years. The same applies to the matter of research which the manufacturer conducts in the laboratory under strictly controlled conditions which he can imitate in his manufacturing plant, while the breeder has to conduct his research over periods of many years under variable conditions on an extremely variable animal product. In the matter of production costs, the manufacturer of artificial fibres is not faced with such factors as droughts and sheep diseases, which are costly to combat and place the farmer at a serious disadvantage.

If the artificial product can advance to the stage where it possesses all the desirable properties of wool, this fact alone should not be sufficient to affect the demand for wool. It will then depend on which fibre is most economically produced and converted into fabric. Even if wool remains the superior product, it could not hold its own if its costs of production are high compared to those of other fibres. It may safely be argued, therefore, that the future of wool will depend on the extent to which it is possible to reduce production costs. This applies not only to the cost of producing the raw product, which is only a fraction of the cost of the finished material, but also to manufacturing costs.

The wool manufacturer's problems are being investigated in his domain but the producer has to bear a great responsibility, since he hands the raw material with its inherent properties over to the manufacturer, and the finished cloth depends to a great extent on the virtues of the raw product.

The results of the present study apply mainly to breeding, which is one of the major factors in wool production, and the discussion which follows will, therefore, be devoted mainly to some aspects of breeding.

For the producer to be able to hold his own in the future, it is essential that he shall know his product, and the means whereby its characteristics are altered. The present investigation comprises a study of the compressibility of wool, but the results obtained are not confined to this attribute alone, for fleece and fibre characteristics are inter-related, and in practice must be considered together.

To the breeder the importance of correlations is two-fold. In the first place, when his breeding policy is directed towards enhancing one characteristic, the values of other characteristics may be altered, a phase of breeding which assumes a special importance in view of the reliance placed by breeders on subjective estimation of wool attributes. In the second place, correlations enable the breeder to assess the relative importance of different fleece attributes and to decide to what extent the value of each of such attributes should be changed in order to enhance the quantity and quality of the fleece most profitably.

On a broad basis the correlations mentioned may be divided into two classes, those existing within individual fleeces, and those existing between different fleeces. An extremely complicating factor is, however, that of selection, for the breeder is able by selective breeding to reduce or enhance an existing correlation or to introduce a new one. As a result, correlations found within one stud may not apply to another stud, and generalisations based on selected groups often have value only for those groups. In the present study an attempt has been made to include wool from as many sources as possible, and the result may, therefore, in some respects be regarded as providing a general background for the intensive study of individual studs and flocks.

In applying the results obtained to breeding, a complication is introduced by the fact that breeders employ terms to denote characteristics not expressible arithmetically. The properties of "harshness" and "softness" have been considered. These terms are self-explanatory, and it only remains to analyse the factors which determine them. Other terms, such as "substance", are less well-defined, and are consequently more difficult to analyse.

Some of the relations found in the present study may be regarded as of purely mechanical origin, and it is proposed to discuss these relations first.

A factor whose effect may be regarded as purely mechanical, is adsorbed water. Speakman (1930) has shown that, whereas the rigidity of wool is reduced in the ratio 15:1 from dryness to saturation, the corresponding change in Young's modulus (by stretching) is only 2.6:1. In the case of cotton, Clayton and Pierce (1929) found that "the effect of humidity is rather less marked on the flexural than on the torsional rigidity". If the compression of the fibre mass is regarded in the light of simple bending of the fibres, the elastic constant involved should be Young's modulus, but in view of the structure of the wool fibre, the value obtained is not likely to be the same as that obtained by stretching the fibre. Even when values obtained with different amounts of adsorbed water are compared on the basis of equal total lengths of fibre, the relative change in compressibility with adsorbed water has been shown to be almost twice that for Young's modulus obtained by stretching. When the values are compared on the basis of equal masses including adsorbed water, the change in compressibility corresponds to that of rigidity. It must be concluded, therefore, that the resistance of the fibre to bending is affected by the reduction, as a result of water adsorption, in the attraction between adjacent micelles, whose long axes are orientated in the direction of the fibre axis. In addition, some torsion of the fibre during bending must be considered as a possibility owing to the twist already present in the fibre and the reversals corresponding to the crests and troughs of the crimp waves (Rossouw, 1931; Woods, 1935).

The considerable difference in the effects of adsorbed water on the rigidity of Young's modulus has been associated by Speakman (1930) with

the large difference between the lateral swelling of the fibre and the increase in length. In this connection he states: "Lateral cohesion between micelles will be reduced to a striking degree by water adsorption and the rigidity of the fibre will suffer a corresponding diminution. Since the micelles are long in comparison with their diameter, the number of breaks between them will be much less frequent along the length of a fibre than along the diameter. The changes in length and strength caused by water adsorption will, therefore, be far smaller than the corresponding changes in cross-sectional area and rigidity".

A possible effect of adsorbed water is also suggested by an observation made by Woods (1935), that the radius of curvature of fibre elements previously immersed in water increases by about 30 per cent. from dryness to saturation. A parallel may be drawn between this phenomenon and the crimping of the fibre. The resistance to compression has been found to diminish when the number of crimps diminishes, and hence with an increase in the radius of curvature of the fibre elements, so that the increase in radius of curvature with adsorption of water may be taken as a contributory cause to the reduction in the resistance to compression of the fibre mass.

The results stress the necessity of performing compressibility measurements under controlled conditions, in view of the fact that an increase of 5 per cent. in the relative humidity, corresponding to an increase of about 1 per cent. in the moisture content of wool, causes a reduction of 12 per cent. in the resistance to compression. This precaution has not been taken by some previous investigators who employed the balloon method. The influence of humidity may also be expected to play an important part in the practical estimation of compressibility by hand, so that while it is possible to compare samples under the same conditions, the comparison of different samples on different days, especially during changeable weather, will be unreliable.

As regards the effect of length, the resistance to compression of the fibre mass evidently depends on the total length of fibre present, and not on the length of individual fibres. Such a conclusion is in agreement with the view expressed in Part I of the study, viz., that during compression the units which bend are not the complete fibres but the elements between adjacent contacts. The possible reduction in the resistance to compression for staple lengths below one inch may be attributed to the increasing number of free ends produced by the separation of elements connected in longer fibres. Attempts to employ still shorter lengths proved unsuccessful owing to the rapidity with which short fibres tend on washing and teasing to develop small lumps, the complete removal of which is practically impossible.

It is recommended that, where possible, sheep experiments involving wool compressibility determinations should be extended over a period sufficient to ensure an adequate length of staple. A length of at least two inches or five cm. is suggested.

Assuming the effects of fibre thickness and crimping to be real, the failure to account for the whole of the differences in the resistance to compression of various wools by means of fibre thickness and crimping, must, in part at least, be due to differences in the elastic properties of the fibres. While admittedly both the mean fibre thickness and the mean number of crimps per inch are subject to sampling errors, these are not sufficiently large to account for the residual variation. As no experimental data are available on the variation in the elastic moduli among Merino wools, the degree to which this factor can account for the residual variation in compressibility cannot be estimated.

There are, however, other important factors which have not been taken into account. One of these is the shape of the crimp wave, for a large number of wave forms occur among fibres, and even along the length of a single fibre (Nathusius, 1866; Barker and Norris, 1930). Another factor is the ellipticity of the fibre cross-section, and the twist present in the fibres, which suggests that during compression the fibres are twisted as well as bent. In this connection it is significant that Barker (1931) states: "Where the trade opinion of the fleeces noted a particularly soft handle, it was subsequently found that the wool of that particular fleece . . . more nearly approached the circular. Although the contour or degree of ellipticity is not the only factor concerned in the production of good 'handle', yet it probably has a decided influence".

In the case of fibre thickness, it was concluded that the fibre thickness has a positive influence on the resistance to compression, but that in general its effect is masked by the crimping. This view seems to be supported by the results of Henning (1934), which showed an increase in resistance to compression with an increase in fibre thickness in the case of wools whose crimping had been reduced by the "Lisseuse" process. That the crimping could not have been completely removed is shown by the further observation that the resistance to compression fell on passing over to the long coarse wools.

In view, however, of the fact that theoretically the fibre thickness has no influence on the resistance to compression, the effect found after removal of the effect of crimping may only be apparent, in the sense that it is caused by other factors correlated with the fibre thickness. For example, factors mentioned above as possible causes of the residual variation may be correlated with fibre thickness, although no data on this point are available.

On the other hand, it is to be noted that if the crimping has an influence on the element length, a point to be considered later, a relation between resistance to compression and fibre thickness may thereby be introduced, for no account was taken of the crimping in the theoretical considerations.

In connection with the effect of fibre thickness, Burns and Johnston (1936) state that "Larose and Winson have both found that an increase in volume under pressure is definitely associated with an increase in fibre thickness". Now the yarns tested by Larose (1934) differed by half a micron in a fibre thickness of 30 microns. In Figure 5 of Winson's (1932) paper are given the pressure-volume curves of two Shropshire samples, according to which the wether sample had a slightly larger ratio of $\frac{v}{v_0}$, and it is stated that the wool of the wether was very slightly coarser, no measurements being being given. On the other hand, according to Figure 7 of the same paper, the comparison of "the Veld wool 70's" and the "fine Cape kid mohair" suggests the very opposite effect, for the 70's wool must have had a considerably finer fibre than the mohair, and yet it had a much greater value of $\frac{v}{v_0}$ at any pressure. The author can therefore not agree with the statement quoted. The present study has suggested that no correlation exists owing to the complicating effect of the crimping. If the effect of the crimping could be removed, the results of the present study would agree with those of Burns and Johnston. Since these authors found, in their own tests, that the coarser wool occupied a greater volume, it is suggested that the

crimping of the wools examined by them was of such a nature that its effect was insignificant, or that the extremely high pressures employed overcame the effect of the crimping, and the fibre thickness alone was operative.

Before considering the effect of the crimping, it must be remarked that the number of crimps was measured on the greasy staple, while the compressional measurements were made subsequent to cleansing in water. The fibres must thereby have lost their original form, but according to Woods (1935), the periodicity in the new forms corresponds to that of the original crimping, though the new forms as observed by Woods would probably not have been completely attained owing to inter-fibre action in the mass.

While it has been stated that the effect of fibre thickness may be only apparent, the same argument is applicable to the crimping. Thus, Barker and Norris (1930) have suggested a mathematical relationship between fibre thickness, number of crimps per inch, and Young's modulus. The possibility must, therefore, be seriously considered that fibre thickness and crimping have no direct effect on the resistance to compression at all, but that they merely indicate the magnitude of other properties which determine the resistance to compression. In the case of the elastic properties, however, if a relationship as exact as that suggested by Barker and Norris exists, the fibre thickness and crimping may be expected to account for a greater, if not the greatest, portion of the variation between samples.

The crimping may also be regarded as an indication of the degree of twist in the fibre, so that the apparent effect of the crimping may be a result of the effect of the twist already present, and possibly also of the ellipticity of the fibre cross-section.

In addition, the crimping may have a real mechanical effect on the resistance to compression. In the first place, the crimping increases the flexural rigidity of the fibre. The finer and more numerous the crimps, the smaller will be the radius of curvature of the fibre elements, and the greater will be the pressure necessary to bend them still further. In this connection an apparent inconsistency must be considered. In Part I of the study, it was deduced, by analogy with a solenoid, that the total volume is proportional to the mean radius of curvature of the fibre elements, suggesting that the coarsely crimped wools should occupy a greater volume than the finely crimped wools, while the present study has shown that in general the opposite is the case. The explanation of the apparent inconsistency lies in the fact that the proportionality between the volume and the mean radius of curvature was deduced on the basis of a constant length of fibre being compressed to different volumes, while the results of the present study are based on the comparison of equal masses of different wools being compressed to the same volume.

In the second place, it is highly probable that the length of a fibre element which bends as a complete unit during compression will be influenced by the crimp wave-length, i.e., that the points of contact between fibres will tend to concentrate at the crests and troughs of the crimp waves. In such an event, the fibre elements will be the shorter, the greater the number of crimps per unit length, and the resistance to compression of the mass will be correspondingly greater. Support is given to this view of the effect of the crimping by the finding that the resistance to compression is not associated with the surface friction of the fibres, for fibre slippage may be prevented by the crimps rather than by the surface friction of the scales.

THE COMPRESSIBILITY OF WOOL.

If the main effect of the crimping is to influence the length of fibre elements which bend as complete units, the question arises in how far the mean element length may be regarded as proportional to the crimp wave-length for a given density of packing. In the ideal case, the lengths of the elements will be fractions or multiples of the crimp wave-length. These lengths will follow some law of distribution, and there will be a value of greatest frequency, to which the mean value will be related. The question then arises as to what factors will determine the most probable and mean values.

In Part I of the study, it was suggested, by analogy with a pile of rods (equation 24) that the mean element length would be proportional to the fibre thickness for a given density of packing. For the mean element length to be proportional to both the fibre thickness and the crimp wave-length, the product of the fibre thickness and the number of crimps per inch must be constant. It follows, therefore, that the mean element length will be the same multiple (or fraction) of the crimp wave-length for all wools which have the same product of fibre thickness and number of crimps per inch.

For other wools, the position will be somewhat different. In the case of a fine-fibred wool with a few crimps to the inch, i.e., a large wave-length, the element length will be smaller, since the fibre thickness is small and the length of fibre large, and the mean element length will be a smaller multiple of the crimp wave-length. In the same way, the mean element length will be a greater multiple of the crimp wave-length when the fibres are coarse and at the same time have many crimps to the inch.

The relation between the crimp wave-length and the estimated element length may be judged from Table 57, where wools following Duerden's standards are considered. In this case twice the element length is likely to be the relevant quantity, representing as it does the distance between two contacts on the same side of the fibre where the forces act in the same direction.

TABLE 57.

Twice the element length as calculated from equation (24) compared with the crimp wave-length of wools which agree with Duerden's standards.

DUERDEN'S STANDARDS.				TWICE ELEMENT LENGTH (cm.).	
Quality No.	Mean Fibre Thickness. (Microns).	Mean Crimps per Inch.	Crimp Wavelength. (cm.).	20 c.c./gm.	10 c.c./gm.
80's.....	17.5	18.5	0.137	0.036	0.018
70's.....	18.4	16.5	0.154	0.038	0.019
66's.....	19.6	14.5	0.175	0.040	0.020
64's.....	20.7	12.5	0.203	0.042	0.021
60's.....	22.2	10.5	0.242	0.045	0.023
58's.....	24.3	8.5	0.299	0.050	0.025

It is seen that for wools whose fineness-crimping relation follows Duerden's standards, twice the mean element length is approximately $\frac{1}{5}$ of the crimp-wave-length at 20 c.c./gm. and $\frac{1}{10}$ of the crimp-wave-length at 10

c.c./gm. The influence of the crimping on the element length is, therefore, likely to be confined to the longer elements, and in the case of a fine-fibred wool with a few crimps to the inch, the effect is probably smaller than with a coarse-fibred wool with many crimps to the inch.

Such considerations suggest yet another reason for the residual variation in the resistance to compression after the effects of fibre thickness and crimping have been eliminated.

The lack of a relationship between the resistance to compression and the surface friction of the fibres at 65 per cent. relative humidity may be due to the fact that the minimum surface friction of the samples examined is sufficient to control fibre slippage, but the cause already suggested, viz., that the crimping is the main factor which controls the slippage, appears more probable. Partial confirmation of this view is given by a comparison of the resistance to compression and the milling shrinkage of a blend. In the case of milling shrinkage, where the surface scale structure is the determining factor, the shrinkage of the blend bears no proportionate relationship to the amounts of the respective constituents (Speakman and Stott, 1931). The resistance to compression of a blend, on the other hand, has been shown to be the weighted mean of the values of the constituents. The comparison suggests that the surface friction is a factor of small moment in determining the resistance to compression.

In either case, the pressure-volume relation of a mass of wool fibres may be expected to differ from that of other fibres which lack the surface scale structure and the crimped form, and in consequence have a greater tendency towards fibre slippage. At the same time the hysteresis between the compression and release curves should be smaller in the case of wool. Pidgeon and van Winsen (1934) were able to explain the reduced resistance to compression of a mass of asbestos fibres with increasing relative humidity by the greater ease of slippage of the fibres over one another, while the present study has shown that in the case of wool, the reduction in resistance to compression may be attributed to an alteration in the elastic moduli of the fibres.

Recently, A. F. Barker (1942) advocated the production of straight fibres, since these should give "a better combing result and a finer count and a stronger yarn, other things being equal". He further regarded crimp "as being simply evidence of variable length of fibre growth". In the present study an attempt was made to procure straight-fibred samples for a study of the effect of fibre thickness in the absence of crimps. No such wools were found, for the so-called straight-fibred wools were found on examination to consist of crimped fibres, and the apparent straightness was due to the fact that the fibres did not combine to form a crimped staple. It was also observed that the crimping of the individual fibres was rather shallow.

Assuming, however, that the production of straight-fibred wools of the Merino qualities is possible, this may be expected to have a pronounced effect on present manufacturing methods, for the crimp serves to some extent as a protection for the fibre when it is stretched during the various manufacturing processes. In this connection Smith (1938) states: "Recent experiments in artificial fibres seem to show that up to a certain point a better spinning result can be obtained (that is to say a finer count and a more even yarn can be reached) by the introduction of a certain amount of crimp". Examination of artificial fibres shows that some types are made permanently crimped and twisted in order to resemble wool.

In view of the existing correlations, straightness of fibre will possibly be accompanied by an increase in uniformity in fibre length, an increase in the circularity of the fibre cross-section, and an absence of twist. It will, however, remove some of the most suitable properties of wool as clothing material, for the crimp structure produces a large number of inter-fibre interstices which are not only partly responsible for the heat insulating properties of wool fabric, but also impart to the fabric a certain sponginess and softness lacking in the more compact structures made up of straight fibres.

From the point of view of the present study, the possible production of straight fibres assumes special importance. The attainment of straightness will presumably be the result of breeding, either for fewer crimps to the inch until they are absent altogether, or for shallower crimps to the limit of straightness. In view of the pronounced effect of the crimping on the resistance to compression, both methods of breeding will tend towards wool with an abnormally low resistance to compression. A possible way of counteracting this tendency would be to increase the fibre thickness, and hence to eliminate the main characteristic which distinguishes Merino wool from other wool. At the same time the harshness will be increased, for the fibre thickness has a greater effect on harshness than the resistance to compression has on this property. If fineness of fibre is maintained, the resulting low resistance to compression will have to be compensated for by a more greasy fleece in order to prevent the fleece from opening up on the sheep and exposing the fibres to the detrimental influences of climate and atmosphere, for it is unlikely that a sufficient fleece density can be attained to prevent this condition. Unless definitely bred for, the amount of grease will probably diminish, since the present study has shown a negative correlation between percentage yield and both resistance to compression and number of crimps per inch.

Another possible effect of the absence of crimp is a reduction in the "springiness" or "loftiness", resulting from a larger hysteresis loop, for with the lack of control of fibre slippage by the crimps, a greater degree of irreversible inter-penetration of the fibres may be expected.

The author is, however, of the opinion that breeding for straight fibres may result in the production of crimped fibres without a crimped staple, unless individual fibres are examined.

In the case of 130 samples from various sources, no relationship has been found between the resistance to compression and the tensile strength. This does not necessarily mean that no correlation exists, for there is evidence to show that certain factors may affect one attribute and not the other. Thus, underfeeding had no effect on the resistance to compression in the experiment described, while the tensile strength of the same samples showed a definite reduction (publication by Bosman and co-workers pending). Further, the average resistance to compression of belly samples equalled the mean for the six regions, while the tensile strength of the belly samples was consistently lower than that of the other regions (van Wyk, 1941). In view of these results, it will be a difficult matter to ascertain whether a real correlation exists between the two attributes.

It is probable that the relationship between resistance to compression, fibre thickness and crimping is one of the most important factors in existing wool practice, and hence in breeding, as the following examples will show.

In his random selection of 1,000 samples, Bosman (1937:1) found that 72 per cent. of the samples showed a divergence from the average relation between the fibre thickness and the number of crimps per inch, and consequently concluded that the estimation of fineness by means of the crimps alone would be in error in 72 per cent. of cases. In practice the crimping does form the main basis of fineness estimation, but wool is usually handled in order to estimate its "quality". Thus Rose (1933) states that all harsh handling wools must be classed down. Whether the resistance to compression or the harshness, or both, are involved in the handling, a high value of either property will, on the basis of the relations found in the present study, indicate too coarse a fibre for the crimping, while a low value of either property will indicate too fine a fibre for the crimping. It is thus seen that the handling of wool in classification serves to correct for the errors in the visual estimation of fineness caused by variations in the fineness-crimping relation.

Another possible result of the effect of the fineness and crimping on the resistance to compression is its bearing on a property known as "substance". According to Mellet (1923), "substance is the power of resistance of the wool, by which it is enabled to stand at right angles to the skin, keeping the fleece closed", while Rose (1930) states that "substance is indicated by fullness of handle, non-compressibility". In view of the above definitions, it is reasonable to regard "substance" as being determined by the factors (1) resistance to compression, (2) quantity and quality of yolk (i.e., grease and suint) and (3) size and density of the staple.

In breeding for "substance", if the breeder is influenced by the resistance to compression, he will tend to breed a coarser fibre than the crimps indicate, although he may not be conscious of the fact, unless he employs means other than the crimping to estimate the fineness of the wool. It will be the policy of the breeder to cull rams lacking this attribute, and the rams retained will bear fleeces which *on the average* have a higher resistance to compression than those of the ewes and wethers. The results of the present study failed to reveal a difference in this respect between the fleeces from rams and ewes in flocks where no selection for "substance" had been practised. The impression that fleeces from rams have more "substance" than those of ewes may thus simply be due to the fact that in practice most rams have been selected partly for this attribute. In this connection it may be pointed out that there is also a belief which has not been experimentally demonstrated that such rams are also good breeders for wool production.

Furthermore, if the rams retained by the breeder have fleeces with a higher average resistance to compression than those of ewes and wethers, the correlations found suggest that their fleeces will also have a coarser fibre than the crimps indicate, compared to unselected material. Such a condition would be a possible reason why Bosman and Botha (1933) found that wool from stud rams was approximately two classes coarser than was indicated by the crimping, while Bosman (1937:1) found an average agreement in his random selection of samples.

With regard to the second factor assumed to determine "substance", viz., the quantity and quality of the yolk, it is also true that selection may give to stud rams a higher average "substance" than that of other animals, but there is the possibility that the fleeces grown by rams and ewes may differ in respect of the yolk, a point not investigated in the present study. However, if in breeding for "substance", the breeder is influenced by the yolk,

of which the grease is the predominating constituent, he may obtain a misleading impression of the resistance to compression and of the density of the fleece. A high correlation exists between the greasy fleece weight and the scoured fleece weight within a stud (Bosman, 1937, 1941), so that breeding for greasy fleece weight will result in an increase in the scoured fleece weight. When the breeder, however, in aiming at "substance" and "density", is misled by the grease and other impurities, he may reduce the yield of the fleeces and so to some extent nullify his attempts at a higher production per sheep.

The negative correlation coefficient found between the clean yield of the fleece and the resistance to compression of the wool suggests that when the breeder, in aiming at "substance" in the fleece is to some extent influenced by the non-wool impurities, he nevertheless tends to produce wool with a high resistance to compression. Alternatively, the correlation found may be the direct result of breeding for "substance" where both the amount of the non-wool portion of the fleece and the resistance to compression of the wool have been enhanced.

No correlation has been found between the yield of the fleece and the fibre thickness, a result in contrast to the findings of Volkmann (1927) and Baumgart (1929). A highly significant negative correlation has, however, been found between the yield and the number of crimps per inch, a result which may be expressed by the statement that the *apparently* fine wools have a lower yield than the *apparently* coarse wools. Since the practical estimation of fineness depends to a large extent on the crimping, the question arises as to whether the correlation found has been introduced by breeding. For, in aiming at "substance", the breeder may tend to produce, firstly, wools with a high resistance to compression, i.e., wools having a coarser fibre than the crimps indicate, and secondly, low yielding wools. He consequently introduces a negative correlation between yield and resistance to compression, and a negative correlation between yield and number of crimps per inch, and removes a possible correlation between yield and fibre thickness. At the same time he regards the crimping as an indication that he is maintaining a reasonable fineness of fibre, which may not be the case.

Now softness of handle, which Rose (1933) associates with "quality", is a desirable property, and it has been shown to be associated with a fine fibre or a low resistance to compression. The attribute of "substance", on the other hand, has in the present study been associated partly with the resistance to compression, which requires either a coarse fibre or a fine crimping. The question arises as to how these two apparently conflicting attributes are to be combined in a single fleece.

It has been shown that for wools whose fineness-crimping relation follows Duerden's standards, the resistance to compression increases with the quality number. Since the harshness is determined largely by the fibre thickness, the increase in resistance to compression with quality number is not accompanied by an increase in the harshness. In the finer wools, therefore, "substance" may be attained by a high resistance to compression without harshness, but with the coarser wools the effect of the fibre thickness in enhancing the harshness must be compensated for by a low resistance to compression, and the "substance" must be attained by other means. Ordinarily this is not produced by an increase in the grease content as shown by the negative correlation between yield and resistance to compression, although it

is a possible method. The third factor suggested as being partly involved in "substance" is probably employed, viz., the size and density of the staple, for Rose (1930) states that "substance is indicated by fullness of handle".

The possible implications of breeding for "substance", viz., a coarser fibre than the crimps indicate, with the consequent difficulty of estimating the fineness, the tendency towards "harshness", and an excessive amount of grease can hardly be considered desirable. Breeding for "substance" may, therefore, be regarded as of rather doubtful value. For a sheep of a certain size, the density of the fleece will be reflected in the wool production per unit length of staple, and provided the latter is satisfactory, and the fleeces do not open up on the sheep, breeding for the attribute of "substance" would seem to be superfluous, and in some respects even undesirable.

The effect of the relationship between fineness and crimping on the resistance to compression, and, therefore, on characteristics estimated by touch, seems to justify a closer examination of the relationship between the two quantities, especially in regard to the standards compiled by Duerden (1929). Measurements on two groups of sheep were compared with the standards by Swart (1937). His method of comparison consisted in relating the difference between the classes as given by the fibre thickness and by the crimping with fibre thickness. The author cannot, however, regard the method employed by Swart as adequate for testing the agreement between a set of observations and the standards, for the following reason.

Suppose the quality number class according to the crimping to be plotted as ordinate, y , against the quality number according to the fibre thickness as abscissa, x . Then the standards will be represented by a line $y=x$. A set of observations which agree with the standards will be symmetrically distributed about this line, and will roughly form the surface of an ellipse whose major axis lies on the line $y=x$. For a given value of x which is smaller than the mean of x , the mean value of y will be above the line, while for a given value of x which is greater than the mean of x , the mean value of y will lie below the line, and the means of y will lie approximately on the regression line of y on x . This is practically similar to the procedure adopted by Swart, except that in his case the mean value of $(x-y)$ was determined for various values of x . The author is of the opinion that such a procedure will give the effect found by Swart, viz., a negative difference between the classes for low values of the fibre thickness and a positive difference for greater values of the fibre thickness, for observations which agree with the standards.

It is suggested that a better test would be to relate the perpendicular distance from any observation to the line $y=x$ with the point where the perpendicular cuts the line. If the values are distributed symmetrically about the line, the sum of the perpendicular distances to any given element of the line should be zero. Since the perpendicular distance from any point (x', y') to the line $y=x$ is given by $\frac{(x'-y')}{\sqrt{2}}$, and the ordinate and abscissa of the point where the perpendicular cuts the line $y=x$ is $\frac{1}{2}(x'+y')$, the procedure suggested is equivalent to relating the differences between the classes with their mean (neglecting the factor $\sqrt{\frac{1}{2}}$).

For this purpose it is convenient to assign an index number to each class, as illustrated in Table 58.

The mean difference between the classes for each value of the mean of the two classes has been calculated for the data of Bosman (1937), Bosman and Botha (1933), Swart (1937), and those obtained in the present study, as shown in Table 59.

TABLE 58.

Quality Number.	Assigned Index Number.
150's.....	1
120's.....	2
100's.....	3
90's.....	4
80's.....	5
70's.....	6
66's.....	7
64's.....	8
60's.....	9
58's.....	10
56's.....	11
54's.....	12

TABLE 59.

The mean difference between the classes as given by fibre thickness and number of crimps per inch calculated for each value of the mean of the classes.

DIFFERENCE BETWEEN CLASSES.

Mean of Class Values.	Bosman (1937) (Random Samples).		Bosman and Botha (1933) (Stud Rams).		Swart (1937) (Experimental Sheep).		Swart (1937) (Veld Grazed Sheep).		Present Study (Various Sources).	
	Fre- quency.	Mean.	Fre- quency.	Mean.	Fre- quency.	Mean.	Fre- quency.	Mean.	Fre- quency.	Mean.
1.5.....	3	+1.0	—	—	—	—	—	—	—	—
2.....	3	0	—	—	—	—	—	—	—	—
2.5.....	—	—	—	—	—	—	—	—	—	—
3.....	24	0	—	—	2	-2.0	1	0	—	—
3.5.....	21	+0.4	—	—	1	-3.0	—	—	1	-1.0
4.....	33	0	1	0	4	-0.5	1	-6.0	—	—
4.5.....	51	-0.9	—	—	5	-1.8	2	-2.0	1	+3.0
5.....	81	-0.4	—	—	5	-0.4	2	-3.0	—	—
5.5.....	60	+0.4	1	+1.0	5	-1.0	3	+1.7	5	+1.0
6.....	87	+0.2	—	—	12	+0.2	10	+1.0	6	-0.3
6.5.....	66	+0.2	8	+0.3	15	+1.1	11	+0.8	25	+0.3
7.....	117	-0.4	5	+0.8	13	+1.5	7	+2.3	32	+0.6
7.5.....	66	+0.3	17	+0.8	24	+2.8	11	+1.5	35	+0.6
8.....	90	+0.4	12	+1.3	14	+2.8	7	+1.7	46	+0.9
8.5.....	84	-0.5	25	+2.3	14	+3.0	1	-1.0	58	+1.1
9.....	51	-0.2	31	+1.6	3	+3.3	2	+1.0	44	+0.7
9.5.....	57	-0.2	12	+1.3	—	—	2	0	31	+1.1
10.....	51	+0.7	8	+0.5	—	—	1	0	19	+1.6
10.5.....	30	0	2	+1.0	—	—	—	—	6	+1.0
11.....	18	0	1	0	—	—	—	—	—	—
11.5.....	9	+1.0	—	—	—	—	—	—	1	+1.0
	1,002	-0.03	123	+1.3	117	+1.5	61	+0.9	310	+0.8
Regression.....	0.0256		0.1405		1.1105		0.4754		0.2148	
	0.89		1.27		15.12		1.91		2.92	

Since the means are based on different frequencies and consequently are not of equal weight, the regression coefficient of the difference between the classes on the mean of the two class values has been calculated for each group, and it is given at the foot of each column. Agreement with the standards will give a zero value for the regression coefficient, and the significance of its departure from zero has been determined by calculating t (Fisher, 1932).

In the case of 1,002 random samples (Bosman, 1937), it is evident that there is no departure from the standards as regards the mean deviation (-0.03) or the slope of the regression line (0.0256). In the case of the stud rams (Bosman and Botha, 1933), there is a mean difference of $+1.3$ from the standards, but the slope of the regression line (0.1405) does not differ significantly from zero, suggesting a general shift of the fineness-crimping relation from that of the standards. The samples from the experimental sheep (Swart, 1937) show a mean difference of $+1.5$ and a marked departure of the regression coefficient from zero (1.1105), confirming Swart's conclusion for these samples, viz., that for high values of fibre thickness the class according to the fibre thickness is coarser than the class according to the crimping, while for the finer wools the thickness is smaller than the crimps indicate according to Duerden's standards. The author cannot, however, agree with Swart that Bosman and Botha's data for stud rams correspond to his own or agree with the conclusion reached in regard to his own results. The samples from the sheep grazed on the veld also show a departure from zero in the regression coefficient (0.4754) but the number of observations is insufficient to render this significant. The results of the present study show a mean shift of $+0.8$ from the standards, and a small but significant difference between the regression coefficient of 0.2148 and zero.

The lack of agreement between his observations and standards led Swart (1937) to question the validity of the standards. Apart from the fact that results obtained on selected groups can hardly be regarded as a fair test of the validity of the standards, the author regards the estimation of fineness by means of the crimps as of extremely doubtful value, and consequently agrees with Swart that the standards in question, or any other standards giving a relation between fineness and crimping, should not be employed in stud breeding practice for estimating the fineness from the crimping.

The standards can, however, serve a useful purpose. Crimping has such a pronounced effect on the compressibility that it must play a considerable part in subsequent processing. Assuming that the experienced sorter may be able to estimate fibre thickness without being misled by the crimping, he has to choose between uniformity in fibre thickness with a variation in the crimping and uniformity in the crimping with a variation in fibre thickness, or he must make a compromise between the two. In any case, variations in the relation between fibre thickness and crimping will result in a lack of uniformity in the sorted lot. To base breeding policy on fibre thickness alone will not be sufficient, and the author is in favour of Swart's suggestion that breeding should be directed towards certain combinations of fibre thickness and crimping. The standards compiled by Duerden are a definite step in this direction, being at present the only link between producer and manufacturer, although they may need revision as the knowledge of the part played by each attribute in manufacture increases.

In breeding for a certain combination of fibre thickness and crimping, certain difficulties will be encountered. In the first place, it is highly probable that fixing certain combinations of fineness and crimping will fix other attributes. For example, as suggested by Table 27, it is possible that fine wools

will then always have a high resistance to compression, and coarse wools always a low resistance to compression. Since all wools at present are in demand, each type being employed for a specific purpose, the reduction in the number of types may meet with some opposition from the manufacturers. With the introduction of such a breeding policy, therefore, the collaboration of the manufacturer is essential. Provisionally the standards can serve as a basis, but the final combinations should be decided upon after the producer has submitted a number of combinations to various branches of the manufacturing industry, and has received the opinions based on actual tests. Should various branches favour different combinations, these could probably be arranged to correspond to the climate and pasturage of the different wool-growing areas, but a difficulty which may be expected when breeding for certain combinations of fibre thickness and crimping is the disturbance likely to be caused by a drought which may upset all the combinations.

The author is, however, of the opinion that the first step of the producer in improving his product is to breed for uniformity, since the manufacturer's methods will thereby be simplified and his production costs reduced. The need for uniformity has been stressed so often that it will not be considered in detail, but it must be borne in mind that at present the artificial product is superior to wool in this respect.

Three types of uniformity may be distinguished, viz., within the staple, within the fleece, and within the flock (Frölich, Spöttel and Tänzer, 1929; Bosman, 1937:2). It is essential that a thorough study should be made to determine whether the fibre uniformity within a staple is correlated with the uniformity among the different staples composing a fleece. Should a correlation exist the breeder's efforts to produce a uniform fleece as judged by hand and eye, methods will tend to produce uniformity among the fibres composing a staple, while the lack of a correlation will necessitate microscopic measurement for determining the uniformity within a staple. Frölich, Spöttel and Tänzer (1929) consider that no relation exists while Duerden and Bell (1931) state that "a high degree of uniformity in quality over the sheep would doubtless be accompanied by less variability in the individual fibres of a staple". None of the authors, however, quote experimental evidence in support of their opinions.

The high correlation between fibre thickness and fibre length found by Duerden and Bosman (1931) and Roberts (1931) suggests that a reduction in the variation in the fineness of the fibres composing a staple will be accompanied by a reduction in the variation in the length of the fibres. According to the present study, however, an alteration in the variability will be accompanied by but little change in the compressibility.

In the case of the fleece, on the other hand, a marked reduction in the variation in resistance to compression between the various regions of the sheep takes place when allowance is made for the variation in fibre thickness and crimping, so that breeding for uniformity in fibre thickness and crimping over the fleeces will tend to reduce the variation in resistance to compression over the fleece.

It is often found that fleeces from one flock are extremely dissimilar, an occurrence which must be ascribed to the lack of a definite breeding policy. Thus it is found that some farmers class their clip into a large number of lines, and while the thorough classing is commendable, the variation in the clip which necessitates such intensive classing must be regarded as highly

undesirable. It is reasonable to assume that for any area there must be a type of sheep and its fleece which is best suited to that area, and every farmer should ascertain which type is most profitable to him, and by careful breeding and selection confine his production to that type only. In this way he will produce his wool most economically, reduce his classing to a minimum, and offer to the manufacturer a uniform type of wool.

While the farmer then should aim at uniformity in his clip and confine himself to the type which gives him the highest financial return per sheep, it is the stud breeder who must bear the responsibility of producing the suitable rams.

A plea for recording in stud breeding has been made by Bartel, Swart and van Rensburg (1936) and by Bosman (1936, 1937:1, 1943), and the breeders themselves appear to be considering the possibility (S.A. Merino Breeders Journal, 1943). While support must be given to such a scheme, too much emphasis cannot be laid on the need for exact methods of determining fleece characteristics. Hand and eye methods have met with a high degree of success in the past, and are responsible for the present standard of South African Merino stud animals, but it is becoming increasingly evident that their effectiveness is rapidly diminishing. Results obtained during the course of the present study, and in other branches of fleece testing in the laboratory, suggest that the main causes of erroneous estimation are the variation in the relation between fineness and crimping, and the presence of the non-wool fleece constituents.

The seriousness of the extent and magnitude of the errors involved cannot be too strongly emphasised, and these errors can be definitely harmful to the interests of the South African producer. For instance, some manufacturers state that an insufficient quantity of "strong" wool (i.e., wool of the coarser qualities) is available for the production of certain classes of goods which they desire to manufacture in the Union. Now the climatic and pastoral conditions of certain areas do not favour the production of "strong" wool, but it can safely be assumed that in a large proportion of cases wools which according to fibre thickness should be classed as "strong" are classed as "medium" or even "fine", owing to the crimping. Such a conclusion is supported by analyses of fleeces exhibited at wool shows and a good instance is afforded by the samples used in the study of harshness, as recorded in Table 33.

Exact measurement in recording will not only be of immense value to the stud breeder himself, but will also aid the farmer in purchasing rams. The stress laid by breeders on "substance" and "bulk", as evidenced by such sayings as "substance fills the bales", is definitely misleading to the purchaser of rams, for too often the "substance" referred to is merely an excessive amount of grease, for which the farmer receives no compensation, and for the production of which the sheep have to be fed.

When a system of fleece recording for rams has been instituted, it is obvious from the work of McMahon (1940) that this alone will not be sufficient. McMahon found that in the case of the Romney sheep, the use of progeny tested sires resulted in an average increase of nearly one pound of wool per sheep in one generation, while the use of sires selected by the usual system required nine generations to produce the same result. Culling 50 per cent. of the ewes each year had little influence, for it was estimated that this method would require 24 generations to raise the average fleece weight by one pound.

Now breeders are well aware that the breeding performance of a ram cannot necessarily be judged by its own fleece, but McMahon's conclusion that only seven ewes are required for the progeny test, suggests that a system, requiring that rams offered for sale to farmers should have been subjected to a progeny test, is feasible. The immense value of such tests to the studmaster himself is obvious.

It is essential that the system of fleece analysis now in operation be extended, and two methods suggest themselves for achieving this purpose. In the first place, the Department should carry out breeding experiments on a much larger scale than hitherto, employing exact methods of measurement for the analyses of the fleeces and for the records of the progeny tests, in order to demonstrate clearly and conclusively the advantages of such a system. In the second place, breeders and farmers should by all possible means be urged to institute such a system in their own flocks and studs.

The general application of a system of fleece analysis may need a more extensive organisation than exists at present, but this cannot be considered an obstacle. While the service has hitherto been available to the farmer free of charge, even a small financial outlay, should this become necessary, would be more than compensated for by the improvement in the flock. In addition, it should be borne in mind that research is partly directed towards the simplification of methods of fleece analysis, and considerable success has been achieved in evolving methods suitable for routine testing.

A system of fleece recording and a definite breeding policy, including breeding for a certain combination of fineness and crimps, will aid in the wider problem of the standardisation of wool. The properties of artificial fibres, such as the fineness and length, are exactly specified as a result of measurement. Wool, on the other hand, is specified by human estimation, and only in the case of the finished, or partly processed, product, is the specification based on measurement. Consequently the manufacturer who buys the artificial product knows exactly what he is receiving, and can select from a large number of types, within each of which the inter-fibre variability is low. The wool manufacturer, on the other hand, having acquired a "lot" nominally classed as one line, has to go to considerable expense in sorting, and then has to blend different types to ensure that his finished product will be reproducible.

The unsatisfactory nature of the present position is being realised, and testing houses have been established in Australia and the U.S.A. While the testing is at present confined mainly to the determination of clean yield, it will no doubt in time to come be extended to other fleece attributes. It is essential that similar steps should be taken in South Africa, if the future of wool production in South Africa is to be assured.

SUMMARY AND CONCLUSIONS.

1. A study has been made of the resistance offered by wool samples to compression at 65 per cent. relative humidity and 70° F. (21.1°C.) temperature. The study has been based mainly on results obtained with the "Pendultex" instrument, designed by Henning (1934), but some additional determinations were made by means of a static cylinder and piston method.

2. A relation has been derived whereby the work done in compressing a wool sample in the "Pendultex" apparatus may be calculated from the number of swings during which the amplitude is reduced from one fixed value to another.

3. During the final constant cycle of compression by the static method, the pressure bears to the inverse cube of the volume a linear relation, which has been written

$$p = A. \left(\frac{1}{v^3} - \frac{1}{v_0^3} \right) \dots \dots \dots (5)$$

With the dynamic method, the law is obeyed by the first compression, and the results follow the relation

$$W = \frac{a_1}{v^2} - a_2 \dots \dots \dots (7)$$

where W is the work done in compressing a sample to a volume v . The coefficient a_1 in equation (7) is an approximation to the coefficient $\frac{A}{2}$ of equation (5), and in the study is taken as the *coefficient of resistance to compression*.

4. The pressure-volume relation is discussed from a theoretical point of view, and it shown that the inverse cube law may be derived on the basis of certain assumptions. An approximate value of Young's modulus by bending can be calculated.

5. An empirical exponential relation between pressure and volume is considered.

6. It is concluded that since the density of packing is not uniform at low degrees of compression, results obtained at low pressures should not be considered together with those obtained at higher pressures, where the density of packing is more uniform and the pressure-volume relation follows the inverse cube law.

7. The method of expressing compressibility and resilience by means of the work done during compression and release is discussed. It is concluded that in the comparison of different wools the work done should be evaluated between volume limits given by equal values of $\frac{v}{v_0}$ for the different wools.

8. A marked reduction in resistance to compression with the adsorption of water has been found.

9. Fibre length has no influence on the resistance to compression down to staple lengths of approximately one inch.

10. No correlation has been found between resistance to compression and fibre thickness. Although this result agrees with theoretical expectation, a highly significant partial correlation coefficient of +0.4330 is obtained when the effect of crimping is allowed for. It has been concluded, either that the fibre thickness has a positive influence which is masked by the crimping, or that fibre thickness is correlated with other factors, besides the crimping, which influence resistance to compression.

11. A highly significant positive correlation coefficient has been found between the resistance to compression and the number of crimps per inch. Possible ways in which the crimping can influence the resistance to compression are discussed.

12. For wools whose fineness and crimping agree with Duerden's standards, the resistance to compression increases with the quality number. Wools which are coarser than the crimps indicate have a higher resistance to compression than wools which are finer than the crimps indicate.

13. A significant partial correlation between resistance to compression and variability in fibre thickness has been found, but the coefficient is probably too small to be an important factor in breeding.

14. No correlation exists between the resistance to compression of a sample and the surface friction of its component fibres. It is concluded that the crimping is a more important factor in controlling fibre slippage during compression.

15. No correlation has been found between the resistance to compression of a sample and the tensile strength of the fibres. There are, however, factors which may influence one of these attributes and not the other, thus masking a possible correlation.

16. Samples presumed to have been selected for specific gravity by a sheep and wool expert were found to have been selected for resistance to compression. It is recommended that the term specific gravity should not be employed in wool practice.

17. Fibre thickness was the main factor to determine the harshness of two sets of samples as subjectively estimated. Resistance to compression and the non-wool fleece constituents were less important, though definite, factors. Harshness is, therefore, determined by the resistance to bending of individual fibres, rather than by the resistance to compression of the mass as a whole. An increase in the surface friction of the fibres is responsible for the increased harshness of alkali treated wool.

18. Dipping wool in a lime-sulphur dip has no effect on the resistance to compression.

19. The variation in resistance to compression over the fleece has been studied and the major part of the variation found to be associated with the variation in fibre thickness and crimping. The results are discussed in relation to sampling in experimental work.

20. There is a highly significant negative correlation between the resistance to compression and the percentage clean yield of the fleece, and a highly significant negative correlation between percentage yield and number of crimps per inch, and no correlation between percentage yield and fibre thickness.

21. No difference in the average resistance to compression of fleeces of rams and ewes could be found. It is concluded that differences observed in practice are due to selection of stud rams for the "substance" of their wool.

22. On the average, the resistance to compression of the wool increases with the age of the sheep for the first four years, and the increase can be associated almost entirely with the increase in fibre thickness.

23. In a feeding experiment, the plane of nutrition had no effect on the resistance to compression of the wool in spite of a marked effect on the fibre thickness.

24. The distribution of resistance to compression is considered, and it is shown that South African Merino wool covers a range of at least 3:1 in this attribute.

25. The bearing of the correlations found on wool practice, with special reference to breeding, is discussed.

26. Possible results of breeding for "substance" are considered, and the desirability of breeding for this attribute is regarded with some doubt.

27. The importance of breeding for uniformity is stressed.

28. Support is given to a scheme of fleece recording in stud breeding, and emphasis is laid on the necessity of employing exact methods of measuring wool characteristics.

29. The establishment of a wool testing house in South Africa is recommended.

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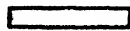


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OF AGRICULTURE**

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 Director of Veterinary Services.

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The Attenuation of Bluetongue Virus by Serial Passage through Fertile Eggs.

By R. A. ALEXANDER, D. A. HAIG and T. F. ADELAAR, Section of Protozoology and Virus Diseases, Onderstepoort.

IN an article by Alexander (in press) details are given of the influence of the temperature of incubation on the multiplication of one strain of bluetongue virus ("Bekker" strain) in fertile eggs. Before the importance of this factor was fully appreciated and serial passage had been reduced to a simple routine, that strain of virus had been passed successively through more than 100 egg to egg passages. It was stated that, by that time, the virus had become so attenuated as to produce little or no detectable clinical reaction in susceptible sheep and consequently the only certain index of infection was the development of a solid immunity to the homologous virulent virus. No opinion could be expressed as to whether this loss of virulence was merely a chance phenomenon, not repeatable with either the same or another strain of virus, whether attenuation took place slowly and progressively with passage or as the result of the sudden appearance of a stable mutant, or whether the lower temperature of propagation was the determining factor. For this reason a second strain of virus was adapted to propagation in eggs at three different temperatures and the virulence for sheep, together with the antigenicity, was tested at regular intervals. The significance of the findings are discussed in the light of the problem of mass immunization in the field.

TECHNIQUE AND MATERIALS.

The technique, conditions of incubation and the temperatures were those detailed in the previous report.

The strain of virus used was that known as "University Farm" (Neitz, in press) since this strain can be relied upon to produce severe clinical reactions in Merino sheep under stable conditions.

A sheep (64506) was destroyed 24 hours after the initial rise in temperature on the 8th day after infection with virulent blood. The spleen was removed with aseptic precautions, passed through a Latapie mincer and desiccated *in vacuo* from the frozen state over anhydrous calcium sulphate, after prefreezing in a dry ice-alcohol bath. The resulting powder was sealed in small glass ampoules in an atmosphere of dry nitrogen and stored at -10° C. to serve as a permanent stock of virus.

The bacteria-free inoculum for the eggs was prepared as a 660 m μ gradocol membrane filtrate of an approximate 2 per cent. broth emulsion of

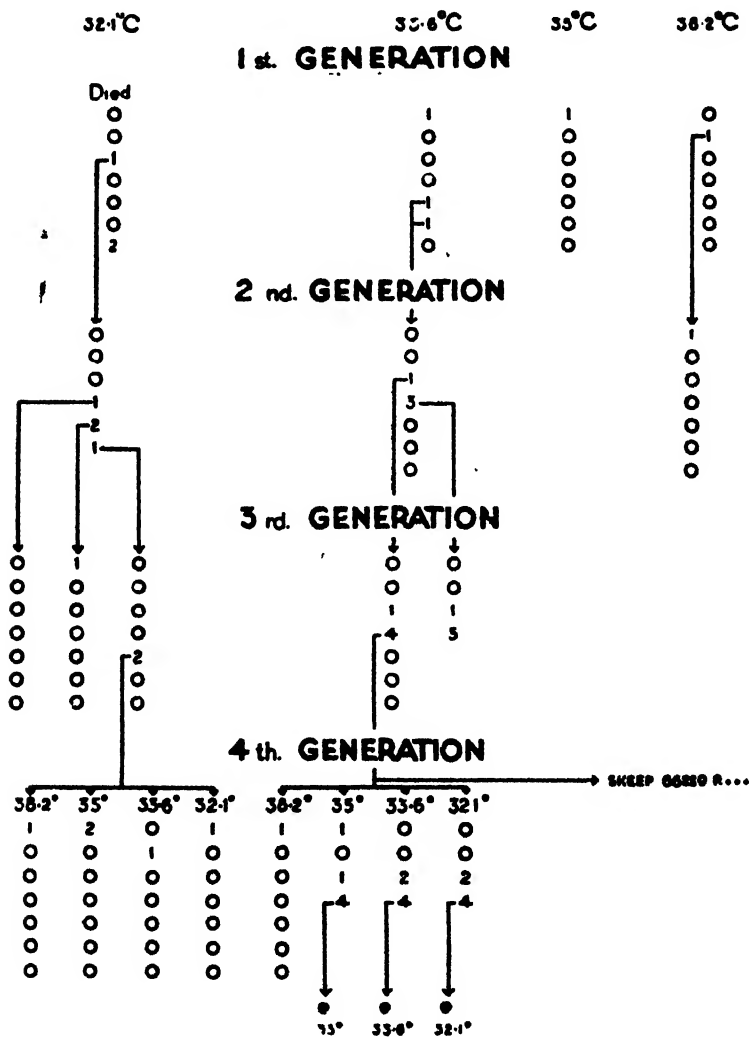
the dried spleen clarified by centrifugation at 3,000 revs. per min. for 2 hours in a Clay Adams angle centrifuge. The virulence of the filtrate was controlled by the subcutaneous injection of 2 c.c. into a sheep (64014). This sheep showed the usual severe reaction (++++) from the 7th to the 12th day after injection.

PROPAGATION IN EGGS.

The procedure adopted for the initial adaptation to eggs is shown schematically in Chart I.

CHART No. I.

Adaptation to Serial Passage in Eggs at 38.2°, 35.0°, 33.6° and 32.1° C.



NOTE.—o=no deaths, numeral=number of embryos which died on that day after injection.

The 24 eggs containing 8 day embryos which had received an injection of 0.1 c.c. of the virulent filtrate were divided into 4 groups of 6 eggs each for incubation at each of the 4 temperatures, viz., 38.2°, 35.0°, 33.6° and 32.1° C. These, and every subsequent group of subinoculated eggs were kept under observation for 7 days after which they were discarded. Death of an embryo was taken as the index of virus multiplication, and subinoculations were made from the dead embryos except those which were found dead within 24 hours; these were assumed to have died of traumatic injury, and were discarded. The results may be summarized as follows:—

A. at 38.2° C.

Only one of the 6 embryos died—on the second day. This was harvested and subinoculated into 6 eggs of which one embryo died within 24 hours.

Result.—No multiplication of virus took place.

B. at 35.0° C.

Only one embryo died within 24 hours.

Result.—No multiplication of the virus.

C. at 33.6° C.

Generation 1.—One embryo died within 24 hours and was discarded.

Two embryos died, one each on the 5th and 6th day. These were pooled, the first after storage overnight in the refrigerator, and subinoculated into 6 eggs to constitute generation 2.

Generation 2.—One embryo died on the 3rd day and 3 on the 4th day. These were harvested as 2 separate groups and each subinoculated into 6 eggs of generation 3.

Generation 3.—One of each group died on the 3rd day, a total of 9 died on the 4th day and the remaining embryo survived for 7 days. Four of the 4th day dead embryos were pooled for subinoculation into 24 eggs of generation 4. A sheep (66259) was given 1 c.c. of this emulsion subcutaneously. It reacted severely from the 5th to the 13th day with clinical lesions of blue-tongue. On immunity test after an interval of 38 days it was proved to be solidly immune.

Generation 4.—The 24 eggs were again divided into 4 groups of 6 each, for incubation at each of the 4 temperatures. At 38.2° C. there were no deaths except one within 24 hours. At each of the other temperatures all the embryos died on the 3rd and 4th day. The 4th day dead embryos were harvested for subinoculation and the strain has been maintained by serial passage at those temperatures until the present time.

Result.—The virus was adapted to propagation in the developing chick embryo by incubation at 33.6° C. for 3 generations. After this initial adaptation the virus continued to multiply when incubated at 32.1° 33.6° and 35.0° C. but not at 38.2° C.

D. at 32.1° C.

Generation 1.—One embryo died on the 3rd day and was subinoculated into 6 eggs of generation 2. Two embryos died on day 7 but on examination were found to be pale and underdeveloped so that death was regarded as non-specific (see previous report) and they were discarded.

Generation 2.—One embryo died on the 4th day, 2 on the 5th day and one on the 6th day. The dead embryos on each day were harvested as groups and each was subinoculated into 6 eggs of generation 3.

Generation 3.—Of the 12 eggs which received emulsion prepared from the 4th and 5th day dead embryos one died within 24 hours and the remaining 11 survived for 7 days when they were discarded. Of the group which were given emulsion from the previous 6 day death, 2 died after 5 days incubation. These were pooled and subinoculated into 24 eggs of generation 4.

Generation 4.—Four groups were again incubated at the 4 temperatures. Four embryos died within 24 hours and one, incubated at 33.6°C ., died on the 2nd day. As no further embryos died the series was discontinued after 7 days.

Result.—Although it appeared as if the virus was becoming adapted to multiplication at 32.1°C . as early as the 2nd serial passage, though this was not confirmed by injection into sheep, no multiplication took place on further subculture.

Comment.—The extreme importance of the temperature of incubation to the adaptation of this strain of virus to multiplication in the developing chick embryo is clearly demonstrated. Using death of the embryo as an index of virus multiplication, and, at the present time there appears to be no other index, the only temperature of the 4 under consideration at which continued multiplication took place was 33.6°C . Had this temperature not been used it might have been concluded, quite justifiably, that the strain of virus could not be adapted to serial egg passage. After the virus had been passed through 3 generations of embryos of 33.6°C . no difficulty was experienced in continuing propagation at that temperature. In addition it could then be cultivated with ease at both 35.0°C and 32.1°C . but no evidence of multiplication at 38.2°C . was obtained. In passing it may be stated that after an additional 50 passages at 33.6°C . a further unsuccessful attempt was made to propagate the virus at 38.2°C .

From the above observations it was concluded that the "University Farm" strain of virus differed from the one previously studied (Bekker strain) in that 33.6°C . and not 32.1°C . appeared to be the optimum temperature for cultivation. As facilities were not available to duplicate the previous detailed investigation, merely the salient features of that work were studied in order to develop a procedure for obtaining the highest titre of emulsion with the greatest economy of material.

While passage was proceeding at each of the 3 temperatures, a record was kept of the fate of all eggs over a period of 4 days after infection. For the sake of brevity only details of generations 11 to 20, 41 to 50, 91 to 100, 121 to 130 are shown in the summary of results in Table 1. These were selected at random as representing early, medium, and late passages, and because the conclusions would not be affected by detailing the whole series.

Result.—A general consideration of the results shows that, throughout the course of the experiment, the technique of handling and injecting the eggs must have been uniform because the number of deaths amongst the embryos during the first 24 hours, i.e., due to traumatic injury, etc., was remarkably constant. Further, there appears to be no significant difference

in the daily mortality at any particular period in either the early or the late passage series with two possible exceptions:—

1. In the series generation 91 to 100 at 33·6 the mortality on the 2nd and 3rd days increased somewhat with a consequent decrease in mortality on the 4th day and survivors beyond that time. This was not repeated in the 121 to 130 generation series so the occurrence was probably fortuitous.
2. The mortality figures for the 3rd day at 35° in the 121 to 130 generation series was low. Since this was compensated by a corresponding increase in the number of deaths on the 4th day, with the number of survivors only slightly higher, this again appears to be merely a chance occurrence.

TABLE 1.

The Fate of Embryos on Passage of Virus at 32·1°, 33·6° and 35·0° C.

INCUBATION.		GENERATION.				TOTAL.
Temp. °C.	Period	11-20.	41-50.	91-100.	121-130.	
32·1.....	1	4-116	9-111	7-112	2-118	22-457
	2	3-113	2-109	0-112	3-115	8-449
	3	51-62	52-57	40-72	44-71	187-262
	4	43-19	43-14	51-21	52-19	189-73
33·6.....	1	4-114	5-114	5-115	6-114	20-457
	2	1-113	2-112	16-99	4-110	23-434
	3	58-55	62-50	81-18	73-57	274-160
	4	32-23	35-15	13-5	24-13	104-56
35·0.....	1	8-111	3-115	10-110	6-114	27-450
	2	3-108	5-110	7-103	2-112	17-43
	3	63-45	57-53	58-45	35-77	213-220
	4	25-20	31-22	12-33	39-38	107-113

NOTE.—4-116, 9-111, etc., means 4 dead, 116 alive; 9 dead, 111 alive, etc.

On the second day there was hardly any significant difference in the number of deaths at any temperature with the possible exception of a slightly increased rate at 33·6° C. It was only during the 3rd and 4th days that differences became apparent. At 32·1° and 33·6°, 376 and 378 embryos respectively died on the 3rd and 4th days, but, whereas the deaths at 32·1° C. were evenly divided between the two days, a significantly larger number died on the 3rd day at 33·6° C. (274 as compared with 104). At 35·0° almost exactly twice as many embryos died on the 3rd day as on the 4th day, and the total number of survivors was significantly higher than at either of the other two temperatures.

Conclusion.—From these figures, using death of the embryo as an index of virus multiplication, it appears that there is little difference in the multiplication at 32·1° and 33·6° except for slight acceleration at the higher temperature. At 35·0° C. conditions for propagation are less satisfactory,

particularly in the case of eggs which survive for 72 hours. In this connection it should be borne in mind that the actual temperatures of these embryos on the 4th day of incubation were on the average 32.37° , 34.13° and 35.54° C. i.e., proportionally higher than the temperature of incubation in each case. (Alexander.)

These results do not throw very much light on the optimum conditions for virus multiplication to that, concurrently, a number of quantitative determinations were made on the virus titre of various emulsions. The general scheme was to take passage material prepared at any one temperature and, as eggs became available in adequate quantities, to titrate that material at each of the 3 temperatures. The results are given in Table 2.

TABLE 2.

The Virus Titre of Embryo Emulsions Cultivated and Titrated at Three Different Temperatures.

VIRUS.		LD (50) AT $^{\circ}$ C.		
Generation.	Cultivated at.	32.1.	33.6.	35.0.
10.....	32.1	4.0000	2.6990	2.0000
13.....		4.7570	3.4770	2.6642
64.....		4.7094	4.9243	2.7477
70.....		4.9208	4.4949	4.0000
64.....	33.6	4.6000	5.0000	4.5925
80.....		5.0000	4.0000	3.5173
68.....	35.0	5.0968	4.8750	4.8561
80.....		5.0000	4.3979	4.0865
132.....	35.0, 32.1*	--	(1) 6.0000 (2) 5.6990	-- --

NOTES.—* 24 hours at 35.0 then transferred to 32.1.

(1) Embryos dead on day 3.

(2) Embryos dead on day 4.

LD(50) calculated according to method of Reed & Muench (1938) and expressed as the logarithm of the 50% death end point.

Result.—Virus propagated at 32.1° C. showed the anticipated decrease in apparent titre as the temperature of incubation for the titration test increased; the decrease was not marked in the case of generation 70. In only one of two experiments with material incubated at 33.6° was the same tendency shown, and with material left at 35.0° this tendency was even less pronounced. Consideration of all the virus titres at 33.6° shows that, after sufficiently prolonged passage to warrant the assumption of full adaptation of the virus to chick embryos, there was no significant difference in virus multiplication no matter at which of the 3 temperatures the eggs were incubated. It is worthy of note, however, that by incubating for 24 hours at 35° and then transferring the eggs to 32° the highest titre emulsions were obtained, with rather more virus in the embryos that died on the 3rd day than those which survived until the following day. Moreover, by adopting that procedure, 16 out of 24 eggs injected died on the 3rd day and all the remainder were dead by the following morning.

Comment.—The result of these two series of experiments do not clear up the question of the most suitable temperature at which to propagate this strain of virus, a finding which is rather remarkable in view of the fact that only at 33·6° was it possible to adapt the strain to multiplication in eggs at all. Comparing these results with the very clear cut results obtained with the Bekker strain of virus, shows that there may be considerable differences, in addition to antigenic structure, between different strains of virus.

ATTENUATION BY SERIAL PASSAGE.

As passage proceeded, material from each successive tenth generation of each series of eggs at the three temperatures was injected into susceptible Merino sheep, the dose being 1 c.c. subcutaneously. Unfortunately there were a few unavoidable omissions but these in no way invalidate the final conclusions. The material used in each case was that used for subinoculation into the next group of eggs so that each sheep received not less than 1,000 M.I.D.'s of virus. It was the original intention to determine the titre of virus in every inoculum by titration in eggs at 33·6° C. From time to time the shortage of eggs made it quite impossible to complete this phase of the investigation. The incomplete results of titration have been omitted since it was early apparent that, within the limits of this method of virus propagation, the severity of the reaction was in no way correlated with the number of M.I.D.'s injected.

The sheep were maintained under stable conditions without exposure to direct sunlight. In addition to carefully controlled daily temperatures they were inspected each morning to estimate the severity of the reactions (c/f. Neitz). The results are shown in tabular form in Table 3.

TABLE 3.
Attenuation of the Virus by Serial Passage through Fertile Eggs.

Egg Genera- tion.	TEMPERATURE OF INCUBATION.		
	32·1 °C.	33·6 °C.	35·0 °C.
3	—	66259. R+++ (+) (6-13)	—
10	—	66228. R++ (7-12)	—
20	66521. R++ (6-9)	—	—
	66519. R+++ (6-9)	—	—
30	66665. R (?) (8-9)	—	66647. R+ (6-10)
40	68659. R+ (6-9) (2)	68948. R++ (6-9) (1)	68715. R+++ (5-10)
50	67604. NR.	68916. R (?) (8-9)	68033. R+++ (4-9)
60	69172. R++ (6-10)	69171. R+ (7-11)	69146. R+++ (6-11)
	69176. R+ (10-13)		
70	69222. R+ (7-8)	69200. NR.	69235. R++ (6-11)
80	70129. R+ (7-10)	69215. R (?) (8)	70056. R++ (+) (5-8)
90	71174. R++ (+) (7-9)	69236. R++ (+) (7-14)	70066. R+++ (7-10)
100	71962. R+ (7-9)	71982. NR.	71986. R++ (6-9)
110	72250. NR.	72071. R(+)	72148. R+ (6-13)
120	72055. R+ (6-7)	72224. NR.	72171. R+ (6-8)
130	72046. R+ (5-7)	72200. R (?)	72023. R+ (5-8)

NOTE.—R+ . . . +++++ indicates varying degrees of severity of reaction.

R+ and R++ denote febrile reaction only.

R+++ and R++++ febrile reaction and clinical lesions. N.R.=No reaction

(1) and (2)—See text on immunity.

6-9, etc Incubation period 6 days, duration febrile reaction 9 days etc.

Results at 32.1° C.—By the 20th subculture it was apparent that some attenuation of the virus had taken place since the reactions produced in two sheep were unmistakably milder than those seen in a number of animals reacting to infective blood. From the 30th generation onward this attenuation was well marked in spite of the fact that one sheep (71174 generation 90) showed a well defined febrile reaction accompanied by slight hyperaemia of the buccal mucosa. The remainder of the sheep either showed no detectable reaction at all or an indefinite fever lasting for not more than 48 hours, without any other symptoms. Further, it was apparent that although the period of incubation remained unaltered, the course of any reaction was considerably decreased.

Results at 33.6° C.—It is unfortunate that generations 20 and 30 were not injected into sheep, more particularly since generation 40 produced a reaction comparable in every respect with that produced by generation 20 at 32.1° C. From then on the reactions were mild and of short duration or practically undetectable.

Results at 35.0° C.—The omission to inject material prior to generation 30 into sheep is of no consequence since every animal which received material from generation 40 to 100 showed marked reactions with well defined mouth and foot lesions. In addition the duration of the reactions were protracted and convalescence was prolonged. After generation 100 a decrease in the severity of the reactions became noticeable so that they approximated those produced by the earlier passage material at the lower temperatures.

Conclusion.—This strain of virus was attenuated by serial passage through eggs. Attenuation took place rapidly on cultivation at 32.1° C. being well pronounced after 30 passages. If attenuation was somewhat slower at 33.6° C. it is not apparent from the somewhat incomplete data presented; the opinion is held that no difference could be determined. At 35.0° C. the virus eventually did become attenuated but a well defined decrease in virulence was not apparent before the 90th or 100th passage. The attenuation showed itself as a decrease in the severity of the febrile reaction, the absence of specific lesions of the buccal mucosa and coronets, a shortened course of any detectable reaction without any adverse sequelae, all without any alteration of the period of incubation.

IMMUNITY.

All the sheep referred to in Table 3 were given an immunity test in the form of 2 c.c. of virulent blood subcutaneously at intervals varying from 14 days to 46 days after receiving egg propagated virus. All were found to be solidly immune, but attention must be directed to two anomalies. Sheep 68948 which received material from generation 40 at 33.6° (marked 1 in Table 3) showed a well defined febrile reaction, without clinical lesions, from the 6th to the 9th day after injection. On application of the immunity test after an interval of 32 days a well marked febrile reaction commenced on the 4th day and lasted for 5 days (maximum temp. 106.8° F.). No clinical lesions of blue tongue were observed and the relation of the reaction to bluetongue remains obscure. Sheep 68659 received attenuated virus generation 40 at 32.1° C., and reacted only with slight fever from the 6th to the 9th day. On immunity test after an interval of 46 days, a severe febrile reaction commenced after 48 hours and persisted for 48 hours.

(Maximum temperature 107.2° F.) Obviously this reaction was not connected with a specific bluetongue reaction and is believed to be something of the nature of "protein shock".

Conclusion.—In spite of the two irregularities quoted it is concluded that whether a sheep shows a clinical reaction or not to the injection of an infecting dose of egg attenuated virus it responds with the production of a solid immunity, which may develop as early as 14 days after injection.

DISCUSSION.

The results of the limited amount of experimental work on the optimum conditions for the propagation of this strain of bluetongue virus are not conclusive. However, two points of extreme importance are apparent, viz.:

1. Using the yolk sac method of infecting fertile hens' eggs after 8 days' preliminary incubation, it was possible to adapt the strain to multiplication in the embryos only by incubation at 33.6° C. Thus the vital rôle played by the temperature of incubation, as previously reported, was confirmed.
2. Adaptation to growth in the embryo occurred rapidly because after only 3 passages at 33.6° C. no difficulty was experienced in starting and continuing propagation at 32.1° C. and 35.0° C., though the range could not be extended to include 38.2° C. This rapid adaptation would seem to be analogous to that reported by Burnett and Bull (1943) from their work on influenza virus.

After the initial adaptation there was little difference in the titre of virus produced by incubation at either of the 3 temperatures, a finding not in accordance with that reported from work with the Bekker strain of virus. It was confirmed, however, that the highest titre of virus together with the greatest mortality on the 3rd day after infection was obtained by incubation at 35.0° C. followed by transfer to 32.1° C. It is apparent, therefore, that a considerable amount of additional work is necessary to elucidate many points on the influence of temperature which, at present, are obscure. This investigation is being carried out in conjunction with the serial passage of other antigenically different strains of virus and will be reported on at a later date.

The observations on the attenuation of the virus are important. In the first place it is shown conclusively that a second strain of virus has been attenuated to a point where it may be injected into susceptible sheep under stable conditions with the production of practically no detectable reaction, followed by the development of a solid immunity to the homologous virulent virus. This attenuation was brought about rapidly, after not more than 20 passages, at 32.1° C., with almost equal rapidity at 33.6° C., but at 35.0° C. it was so retarded that full attenuation was obtained only after about 100 passages. No opinion whatever can be expressed on the mode of action of the temperature of incubation in the production of avirulence. It is reasonable to assume, however, that, if it has been possible to attenuate two strains, it will be possible to attenuate more of the antigenic variants that have been recognized and isolated recently. This assumption is being borne out by the work at present under way, and when considered in conjunction with the solid immunity production, has an important bearing upon the whole problem of the control of the disease in the field.

Neitz showed that the method of immunization at present in use has two major defects:—

1. The attenuated, but comparatively avirulent natural strain of virus, which constitutes the essential portion of that vaccine, produces severe reactions in the field and under unfavourable climatic conditions may be the cause of mortality and serious economic loss.
2. The use of a single strain of virus, with a narrow antigenic range, results in the production of an immunity which is inadequate to protect against the plurality of virus strains which are now known to exist.

There remains a great deal more work to be carried out on the polyvalent immunity produced by a combination of two or more strains of egg attenuated virus, but even at this early stage it is apparent that the above two major defects may be eliminated. It only remains to indicate the possibility of using the developing chick embryo to produce the large quantities of vaccine that are required annually.

At present rather more than $2\frac{1}{2}$ million doses of vaccine are issued each year, and of that amount rather more than half during the 3 months period October to January. With the introduction of a safe and more efficient product it is estimated that the demand would be approximately doubled so that provision would have to be made for the production of at least 5 million doses per annum. Fortunately the virus possesses remarkable keeping qualities on storage at $\pm 5^{\circ}\text{C}$. so that provision could be made to meet the peak demand during the spring or early summer by continued production throughout the year and storage either in bulk or as the final product. It has been shown that under the conditions described it is possible constantly to produce embryo emulsions with a titre of not less than 10^{-6} , and it is known that 10 c.c. of emulsion is obtained from 3 embryos. If the emulsion is diluted 1 to 500 then 6 eggs would be required to produce 100 litres of vaccine containing 200 M.I.D.'s of virus per dose of 1 c.c. That means that the entire annual output of monovalent vaccine could be obtained from 3,000 embryos which would necessitate the injection of not more than 12 eggs per working day. Naturally additional fairly large numbers of eggs would be required for the quantitative determinations which are inseparable from routine vaccine production, but it is confidently believed that the entire task could be carried out by two trained technicians provided with very simple equipment and accommodation.

There still remains some research to be carried out to determine such points as the most suitable diluent to serve as a vehicle for the virus, the most suitable bacterial preservative and the keeping qualities of the final vaccine under field as well as laboratory conditions. These, however, are comparatively minor, though important points, upon which considerable data have been collected so that it is believed that the production of an efficient vaccine against bluetongue is in sight.

SUMMARY.

1. A strain of virus (University Farm strain) was adapted to propagation in the developing chick embryo by incubation of infected eggs containing 8 day embryos at 33.6°C . but not at 32.1°C ., 35.0°C . or 38.2°C .

2. After 3 serial passages at 33.6° C. it was possible to continue propagation at 32.1° C. and 35.0° C. but not at 38.2° C.

3. Using death of the embryos as an index of multiplication of egg-adapted virus there was little difference in the results obtained from incubation at 32.1° C. or 33.6° C. except that multiplication was slightly retarded at the lower temperature. At 35.0° C. the number of survivors beyond the 4th day of incubation was significantly increased.

4. There was little variation in the titre of emulsions produced from dead embryos at either of the temperatures after adaptation to eggs by serial passage.

5. The highest titre emulsions (not less than 10^{-5}) together with the highest death rate on the 3rd day were produced from eggs incubated for 24 hours at 35.0° C. and then transferred to 32.1° C.

6. The virulent strain of virus was attenuated by serial egg to egg passage. At 32.1° C. attenuation took place rapidly after approximately 20 passages, at 33.6° C. at approximately the same rate, but at 35.0° C. it was delayed until about the 100th subculture.

7. Whether the attenuated virus produces a clinical reaction or not a solid immunity is produced against the homologous strain of virus.

8. The application of the results to the production of large quantities of vaccine for the mass immunization of sheep in the field is discussed.

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Studies on Immunity in Heartwater.

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INTRODUCTION.

FROM the results of a series of experiments on the duration and nature of immunity to heartwater in sheep it was concluded (Neitz, 1939) that:—

1. since there is complete cross-immunity between ten strains of "virus" it is possible that all strains are antigenically similar; at least no plurality of virus strains has been demonstrated up to the present;
2. recovery from heartwater is followed by a temporary period of premunition, when "virus" may be detected in the peripheral circulation for periods up to sixty days;
3. the period of premunition is followed by a period of sterile immunity during which the degree of immunity declines at a rate which varies within very wide limits in different sheep;
4. reinfection during the period of partial immunity initiates a repetition of the cycle-premunition, sterile immunity, partial immunity.

These results are of considerable practical importance to the whole problem of mass immunization in the field, but were based upon meagre data for periods longer than two years after immunization, so that arrangements were made for a repetition or extension of the work.

EXPERIMENTAL PROCEDURE.

Merino sheep which had recovered from heartwater in a variety of experiments were confined in a small camp together with a number of susceptible controls. Daily temperatures were not taken, but the animals were under regular observation and no illness was detected; there were no deaths from heartwater among the controls so that it is safe to assume that the animals were not exposed to natural reinfection while in the camp. In May 1942 all the sheep were transferred to the experimental out-station at Bestersput in the Free State where heartwater does not occur. From May 1942 until they were returned to Onderstepoort in February 1944 for immunity tests, it is certain that there was no exposure to natural heartwater. All the immunity tests were carried out in the sheep stable under the ordinary conditions of this type of experimental work.

Chief characteristics of the strains used.

1. The "Mara" strain was isolated from naturally infected sheep that were exposed on the government experimental station at Mara near Louis Trichardt, in the Northern Transvaal. The inoculation period in sheep is seldom less than seven days and usually about eleven days. It is a comparatively avirulent strain, as seen from the fact that during the period May 1940 to February 1944, out of 240 sheep used for maintenance of the virus by serial passage, 50 (= 24 per cent.) recovered without treatment.

2. The "Salem" strain was isolated from a naturally infected calf at Salem in the vicinity of Grahamstown in the Eastern Province. It is characterized by an incubation period somewhat longer than that of "Mara" and is rather less virulent. It was maintained for only a limited number of passages through sheep so that the figures of percentage mortality are of little significance.

3. The "Schalekamp" strain was recovered from naturally infected Merino sheep, that became infected on the farm Bankfontein close to Middelburg in the Eastern Transvaal. Its chief characteristics are an exceedingly short period of incubation (about six days), a rapid course, frequently lasting not more than 48 hours, and 100 per cent. mortality with the presence of enormous numbers of rickettsias in intima preparations of the jugular veins.

There is complete cross-immunity between these strains of "virus" providing the immunity tests are carried out within two months of defervescence of the febrile reaction.

Immunity Tests.

The test dose of virulent heartwater blood was 10 c.c. citrated blood given intravenously; the virulent blood was obtained from sheep at the height of the febrile reaction to the indicated strain in routine passage experiments. The temperatures of all sheep were checked daily. Two of the controls used had been running with the sheep at Onderstepoort and at Bestersput throughout the course of the experiment and were found to be fully susceptible. Full details of the results are given in the appendix while a summary of the significant features are given in Table I.

Results.—If the appendix is consulted in conjunction with Table I it will be noticed that three sheep were immune to the "Salem" strain and nine to the "Mara" strain when they were given an immunity test of "Mara" virus one or two months after the immunizing injection. The twelve sheep were solidly immune. For the purposes of the investigation into the duration of immunity the interval is calculated from the date of the injection for the immunity test. Incidentally this, together with the immunity tests on the two sheep after intervals of 46 and 48 months, where the "Schalekamp" strain was used, is additional evidence on the antigenic identity of all the strains examined up to the present time.

From Table I it is seen that there is a gradual but progressive decrease in the degree of immunity with the passage of time as shown by the increasing proportion of sheep which reacted to the immunity test. This becomes more apparent on consulting Table 2, which summarizes the combined results of Table I and those similar tests after shorter intervals previously reported by Neitz (1939). Apart from the one sheep which died

TABLE 1.
Duration of Immunity to Heartwater.

Number of Sheep.	Interval Months.	Reaction.	Result.
1	31	—	1 Immune.
1	32	10/6.....	1 Reacted.
1	33	—.....	1 Immune.
3	34	— 7/9, 13/3.....	1 Immune, 2 Reacted.
3	35	— 11/6 11/7 *.....	1 Immune, 2 Reacted.
3	36	8/8 11/7 11/6.....	3 Reacted.
5	38	— * 15/2 13/2 10/7 *.....	2 Immune, 3 Reacted.
1	39	—.....	1 Immune.
1	41	—.....	1 Immune.
2	42	11/6 10/7.....	2 Reacted.
1	43	11/7.....	1 Reacted.
2	44	15/8 * 12/4.....	2 Reacted.
1†	46	9/11*	1 Reacted.
1†	48	8/6.....	1 Reacted.

In the table the numerator = period of incubation, the denominator = duration of febrile reaction.

* Strain "Schalekamp" for immunity test. All others strain "Mara".

† Indicates sheep from which subinoculations were made; the results are given in Table 3.

TABLE 2.
Summary of all tests on the duration of immunity.

Interval, Months.	Number of Sheep.	Immunity Test.		
		Immune.	Reacted and Recovered.	Reacted and Died.
1-6.....	40	40	0	0
7-12.....	24	22	1	1
13-18.....	19	17	2	0
19-24.....	23	22	1	0
25-30.....	9	6	3	0
31-36.....	13	7	6	0
37-42.....	12	5	7	0
43-48.....	5	0	5	0
49-54.....	2	2	0	0
55-60.....	1	1	0	0
1-60.....	148	122	25	1

in the 7 to 12 months group, all the reactors showed only a febrile reaction of varying severity and duration, with or without some inappetence, but no clinical symptoms of heartwater whatsoever. From a clinical point of view an aetiological diagnosis could not have been possible in a single instance. No explanation can be offered for the death of the sheep in a group where the immunity should have been solid, but isolated instances of the failure of individual animals to develop an immunity have been encountered before.

A point of interest is the fact that it was possible by an *in vivo* test, to get some idea of progressive decrease in immunity. As a general rule where the period of incubation was short the subsequent reaction was greater. For instance where the temperature did not rise until the 13th or 15th day the subsequent reaction was very mild and lasted for only about 48 hours (c.f. 34 and 38 month groups) whereas where the temperature rose earlier the reaction was much more severe (c.f. later groups).

Briefly the results may be summarized by stating that, in sheep, immunity to heartwater as a result of recovery from infection is solid for at least 12 months after which there is a gradual decrease, but a very considerable degree of immunity exists for periods up to four years, at which time the immunity is still sufficient to protect against fatal infection.

In the previous report (Neitz, 1939) attention was directed to the extreme importance of determining whether reactions, characterized by only a fever and no pathognomonic clinical symptoms, were in fact specific reactions to heartwater virus and not to some other inadvertent contaminant. Consequently a series of subinoculations into heartwater immune and susceptible sheep were made as detailed in Table 3. Initially all the sheep were immune to strain "Mara" except one (59069) which had recovered from infection with "Salem" strain virus. For the immunity tests "Mara" strain was used except in two sheep (64165 and 68744) which received the "Schalekamp" virus. The test dose of virus was 10 c.c. given intravenously but for the subinoculations 20 c.c. of fresh citrated blood was used. All the non-reactors in the subinoculation tests subsequently were found to be susceptible to heartwater.

Results.—A number of most important results emanated from this experiment:—

1. Four sheep, which had recovered from infection between 34½ and 44 months previously, showed febrile reactions of varying degrees of severity when subjected to an immunity test and all recovered. Subinoculation of blood drawn during the febrile reaction produced heartwater in six susceptible sheep (5 died and 1 recovered) and no reaction in three sheep known to be immune to heartwater.
2. One sheep which had recovered 37½ months previously showed no reaction on immunity test. Subinoculation of blood drawn at a time when a reaction could have been anticipated, that is on the 13th day after injection, produced heartwater in a susceptible sheep and this sheep died.
3. Two sheep which had been immunized one and two months previously were solidly resistant to an immunity test but fully virulent virus was detectable in the blood on the 15th and 25th day after injection.

TABLE 3.

IMMUNITY TESTS.						SUB-INOCULATIONS.				
Number of Sheep.	Interval. Months.	P. of I., Days.	Duration. Days.	Result.	Interval. Days.	Number of Sheep.	History.	P. of I. Days.	Duration, Days.	Result.
57191	44	15	8	Reacted	18 18	69033 69050	Susceptible Susceptible	12 12	6 4	Died. Died.
59076	42	10	7	Reacted	13	69026 69034	Susceptible Immune	10 —	16 —	Recovered. No Reaction.
58773	2 38	— 10	— 7	No Reaction Reacted	— 13 13	— 69075 69118	— Susceptible Immune	— 9 —	— 13 —	— Died X. No Reaction.*
59358	1½ 37½	— —	— —	No Reaction No Reaction	— 13	— 69113	— Susceptible	— 11	— 5	— Died.
59069	1½ 34½	— 11	— 7	No Reaction Reacted	— 13 13	— 67450 67423	— Susceptible Immune	— 7 —	— 8 —	— Died. No Reaction.*
64165	8	—	—	No Reaction	0 11 13	68997 69061 68744	Susceptible Susceptible Susceptible	— 13 —	— 4 —	No Reaction. Died. No Reaction.*
67423	1 2 3 5	— — — —	— — — —	No Reaction No Reaction No Reaction No Reaction	— — — 13	— — — 68539	— — — Susceptible	— — — —	— — — —	— — — No Reaction.
69118	1½ 2	— —	— —	No Reaction No Reaction	— 13	— 67962	— Susceptible	— 15	— 6	— Died.
68744	1	—	—	No Reaction	14	69159	Susceptible	25	3	Died.

* See subinoculation below.

Died X = Died with demonstration of *R. ruminantium* in intima smears = Heartwater.

P. of I. = Period of Incubation.

4. One sheep failed to show any reaction to immunity tests applied on three successive occasions at monthly intervals. Two months later a fourth immunity test was given; there was again no reaction and no virus was found to be circulating on the 13th day by subinoculation into a susceptible sheep.
5. Blood was subinoculated from one sheep which received an immunity test after an interval of eight months. No virus was present in the blood immediately before the immunity test or on the 13th day afterwards, but blood drawn on the 11th day produced a fatal case of heartwater in a susceptible sheep.

CONCLUSION.

From the results of this series of experiments it may be concluded:—

1. The febrile reactions indicating a partial break-down in immunity were due to heartwater "virus".
2. Fully virulent "virus" may be expected to circulate in the peripheral blood of immune sheep after injection at that time when it would be found in a reacting susceptible sheep, no matter whether a detectable reaction is produced or not.

DISCUSSION.

The previous report of gradual decrease in the degree of immunity in sheep which have recovered from an artificially induced attack of heartwater has been confirmed, but it has been shown that after a lapse of four years this immunity is sufficient to protect against fatal infection under laboratory conditions. Since it has been shown that in the majority of cases virus has disappeared from the peripheral blood by the end of the febrile reaction, though in exceptional cases it may persist for up to 60 days, the conception of immunity in heartwater as a premunition followed by a gradually diminishing sterile immunity appears to be acceptable. The most important observation, however, is the record of circulating virus following reinfection during the period of sterile immunity, even though the immunity may be sufficiently high to block out any reaction completely, thus indicating multiplication of rickettsias in an immune or partially immune animal. Apart, entirely, from the interest and theoretical importance of this observation in general, it is of immediate practical importance to the whole problem of the control of heartwater in the field. It has always been difficult to explain the continued presence of infection in a high percentage of ticks on any given farm on the assumption that larval and nymphal ticks of the genus *Amblyomma* became infected only by feeding on a reacting animal or for a short period after recovery. Still more difficult was it to explain the almost immediate incidence of heartwater amongst susceptible animals introduced on to farms when there had been no cases of heartwater for considerable lengths of time. This has led to the belief that some reservoir of infection, other than the susceptible domestic ruminants, played a major rôle in the maintenance of infection in the tick population. While such reservoirs may still await identification, it has become quite evident that the mere presence of sheep and presumably also cattle is entirely adequate for the maintenance of infection, and the rôle of any other reservoir may be quite insignificant.

The fact that the immune animal itself constitutes the reservoir of infection necessitates a modification of views on the methods of control. Elimination of the susceptible host by mass immunization will control mortality but

cannot assist in eliminating the disease. Total eradication can only be achieved by elimination of the tick, and, at least under present conditions in South Africa, this is not possible. The only solution is the development of a practical method of immunization. There is one consoling feature, namely that search for such a method need not be confined to a field dependent upon the use of an inactivated virus, because use of an active, though possibly attenuated vaccine, can have no material effect in increasing the infection in a given area.

It is necessary to point out that the observations have been made on sheep. It is essential that similar work should be carried out on cattle.

SUMMARY.

1. It has been confirmed that the immunity in sheep following recovery from infection comprises a short period of premunition, followed by a period of gradually decreasing sterile immunity.
2. Infection during the period of sterile immunity again initiates the cycle premunition, sterile immunity.
3. Circulating virus is detectable in the peripheral circulation of immune sheep following reinfection, whether a demonstrable reaction is produced or not.
4. Up to a period of four years after recovery—the limit of the experiment—the residual immunity is sufficient to protect against fatal infection in sheep under laboratory conditions.
5. The significance of these findings and their relation to the problem of the control of heartwater are discussed.

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Number of Sheep.	First Immunity Test.				Second Immunity Test.				Remarks.
	Injected with Heartwater Strain.	Incubation Period in Days.	Result.	Interval in Months between Infection and Immunity Test.	Injected with Heartwater Strain.	Incubation Period in Days.	Duration of Disease in Days.	Result.	
58896	" Mara "	14	Recovered	31	" Mara "	—	—	—	—
59218	"	6	"	32	"	10	6	Recovered	—
58005	"	6	"	33	"	—	—	—	—
58346	"	8	"	34	"	—	—	—	—
59204	"	8	"	35	"	11	6	Recovered	—
59186	"	9	"	36	"	8	8	Recovered	—
59284	"	8	"	39	"	—	—	—	—
58775	"	8	"	41	"	—	—	—	—
59082	"	11	"	42	"	11	6	Recovered	—
59076	"	9	"	42	"	10	7	Recovered	—
58776	"	7	"	43	"	11	7	Recovered	—
57191	"	8	"	44	"	15	8	Recovered	Blood subinoculated into Sheep Table 3.
58444	"	7	"	46	" Schlabkamp "	9	11	Recovered	Blood subinoculated into Sheep Table 3.
54280	"	12	"	48	"	8	6	Recovered	—
58890	" Salom "	11	"	1	" Mara "	—	—	—	—
58927	"	12	"	1	"	—	—	—	—
59069	"	7	"	1	"	—	—	—	Blood subinoculated into Sheep Table 3.
59146	" Mara "	9	"	1	"	—	—	—	—
59273	"	9	"	1	"	—	—	—	—
58630	"	10	"	1	"	—	—	—	—
58788	"	8	"	1	"	—	—	—	—
59129	"	8	"	1	"	—	—	—	—
59347	"	9	"	1	"	—	—	—	—
58358	"	6	"	2	"	—	—	—	Blood subinoculated into Sheep Table 3.
58773	"	6	"	2	"	—	—	—	Blood subinoculated into Sheep Table 3.
57020	"	5	"	2	"	—	—	—	—
58321*	Control	—	—	—	—	—	—	—	Reinoculation.†††
58001*	"	—	—	—	—	—	—	—	Reinoculation.†
68706	"	—	—	—	—	—	—	—	Reinoculation.†
68797	"	—	—	—	—	—	—	—	Reinoculation.††

† = very rare; †† = rare; ††† = fairly frequent.

* Control sheep kept with recovered sheep at Onderstepoort and Besterput.

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Ticks in the South African Zoological Survey Collection.

Part VI.—Little Known African Rhipicephalids.

By GERTRUD THEILER, Section of Parasitology, Onderstepoort.

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LIST OF AFRICAN RHIPICEPHALIDS WHOSE DESCRIPTIONS ARE NOT INCLUDED IN THIS ARTICLE.

- R. appendiculatus* Neumann 1901.
R. duttoni Neumann 1907 may prove to be a synonym.
- R. ayrei* Lewis 1933.
 Parasitology XXV, p. 269.
- R. bursa* Canestrini and Fanzago 1877.
 Occurs in North Africa.
- R. capensis* Koch 1844.
R. longus Neumann 1907 is to be considered a variety of *R. capensis*.
 Synonym. *R. sulcatus* Neumann 1908.
- R. eretsi* Neumann 1897.
- R. eretsi mimeticus* Dönitz 1910.
 Syn: *R. eretsi-albigeniculatus* Warburton 1916.
- R. glabroscutatum* du Toit 1941.
 Onderstepoort Jnl. Vet. Sc.-Aml. Ind: XVI p. 115.
- R. lundbladi* Zumpt 1942.
 Recorded from Madeira and placed in the *appendiculatus* group.
 Z. parasitk. XII p. 538.
- R. macropis* Schulze 1936.
 (From Aden, a variety of *R. sanguineus*?)
 Z. Parasitenk VIII p. 521 figs.
- R. maculatus* Neumann 1901.
 Syn: *R. ecinctus* Neumann 1908. (Zumpt makes *ecinctus* a synonym of *R. pulchellus*).
 See Warburton 1932 *Parasitol*: XXIV p. 567.
- R. neavei* Warburton 1912.
 Parasitol. V, p. 9.
- R. neavei* var. *punctatus* Warburton 1912.
 Parasitol. V, p. 10.

R. oculatus Neumann 1901.

Var. *R. pravus* Dönitz 1910.

Warburton 1912 thinks *R. pravus* may be the same as *R. naevei* var. *punctatus*. Zumpt 1942 places it in the *appendiculatus* group.

R. pulchellus Gerstäcker 1873.

Syn. R. marmoreus Pocock 1900. See Cunliffe *Parasitol.* VI p. 210.

Var. *humeralis* Rondelli 1926.

R. sanguineus Latreille 1806.

R. simus Koch 1944.

Pterygodus fulvus Neumann 1913.

From North Africa.

See Colas-Belcour. *Arch. Inst. Past. Tunis* p. 430 figs.

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BEDFORD (1932). Key and check list of S. African Ectoparasites. 18th *Report Dir. Vet. Serv. and Anl. Ind., Union of S. Africa*.

BEQUAERT (1931). Synopsis des Tiques du Congo Belge, *Rev. Zool. Bot. Afr.*, Vol. XX, No. 3, p. 209.

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NUTTALL (1916). Ticks of the Belgian Congo. *Bull. Ent. Research*, Vol. VI, No. 4, p. 313, figs.

THEILER, G. (1942). Ticks off domestic stock in Portuguese East Africa, *Moçambique documentaria trimestral. Lourenço Marques*, Vol. XXXIII, pp. 51-120, figs.

INTRODUCTION.

Frequently the re-editing, recapitulating and the assorting of known facts lead to an increase in knowledge. With this in mind, and in view of the renewed interest which is being taken in the more precise distribution of African ticks, it has been deemed advisable to make available to zoologists and to field veterinarians the descriptions of some of the lesser known African ticks; descriptions scattered during the last forty years in various journals which are not generally available. Nuttall *et al* rendered a great service by issuing monographs on the ixodid genera *HAEMAPHYSALIS*, *IXODES* and *AMBLYOMMA*, but the monograph on the genus *Rhipicephalus* still has to be written. Zumpt 1942 has written a series of Articles under the title of "Vorstudie zu einer Revision der Gattung *Rhipicephalus* Koch". published in *Z. Parasitenk* XI and XII. This work, reviewed in the *Vet. Bulletins* XII and XIII, unfortunately is not procurable at the moment.

REMARKS ON THE CLASSIFICATION OF THE GENUS RHIPICEPHALUS.

Diagnostic Characters.—Usually inornate; palps short; eyes present; basis capituli usually hexagonal dorsally; hypostome 3/3; all coxae with two spurs; tarsi tapering, with two successive ventral terminal spurs; festoons present; the males possess an anal shield, an accessory anal shield may be present or absent; stigma in the male an elongate comma, in the female more compact.

The genus itself is very characteristic and easily recognizable, but the identification of its species is difficult, often extremely difficult. Most rhipicephalids are inornate, so that one cannot rely on *ornamentation* as a confirmatory feature. Then again the *coxae* also resemble one another throughout; or, if there are any variations within the genus, these variations are so inconspicuous as to be negligible, so another classificatory feature falls away. The *legs* too are built to the same plan, with their tarsi tapering gradually and having two terminal spines ventrally, and with their caruncle large; generally the legs are medium in size, occasionally the fourth pair may be enlarged as in *R. appendiculatus*; but usually there is no marked nor appreciable difference between them. So that neither the *coxae* nor the tarsi can be used for purposes of specific identification.

The only features of any classificatory value whatsoever are, in order of importance, (a) *in the male*; the conscutum, the anal plates, the basis capituli and the palps; (b) *in the female*; the scutum, the basis capituli and the palps. The size of the specimen may be of some assistance; some species are small; the size of any one species, unfortunately, varies enormously, so that frequently a large species is represented by small individuals; fortunately, however, though a small species can be represented by yet smaller individuals, it will never produce large ones. Variations are also shown by other features used in the identification of the rhipicephalids. Thus the *punctations* though conforming to a certain pattern, may yet vary considerably within that pattern, so that no two individuals will show an identical picture. The range in size, in depth and in distribution of the punctations may be quite great, but the sum total effect yet falls within the typical pattern. The *anal plates* may show slight variations within the species, but on the whole are perhaps more constant in character than are the punctations. In the structure of the *palps*, also, the interspecific differences are but slight, but they do appear to be the most constant and the least variable of the classificatory features; unfortunately, however, but few workers thus far have paid any attention to them.

Hence, in republishing the descriptions of the lesser known rhipicephalids, only those features which are of specific value have been selected from the original articles and the more general features have been studiously omitted. Descriptions of species represented in the Onderstepoort collection have been enlarged and brought up to date, and where available the larva and the nymph has also been described. No effort, however, has been made to evaluate the validity of those species not seen by the author, nor is any opinion expressed on the affinities of any of these species.

" RHIPICEPHALUS ARMATUS " Pocock 1900.

Male (Figs. 1 and 2).

5 mm. × 4 mm. Uniformly deep brown; slightly convex, shiny. *Conscutum* twice as broad posteriorly as anteriorly. Eyes, flat, marginal. Cervical groove deep, wide, slightly convergent posteriorly. Lateral groove deep, extending from the eye to the anterior edge of the last festoon, strongly pitted. According to Pocock: "posteriorly there is a pair of deeply pitted grooves, and between them and the lateral groove on each side another similar but curved groove which extends from in front of the middle as far back as the end of the lateral groove." Punctuation: in addition to the punctures in the lateral grooves, there are a few coarse punctations scattered here and there". According to Neumann: "Punctations very large, deep.

contiguous or almost contiguous in the lateral grooves, continuing the lateral grooves anteriorly, and forming over the rest of the scutum irregular lines somewhat as in *R. simus*. In the largest individuals these linear punctations are in grooves of which there are two short posterior internals, and two externals, somewhat concave and twice as long. (The usual posterior median and posterior lateral grooves appear to be obliterated by the above-mentioned two pairs of heavily punctated lines).

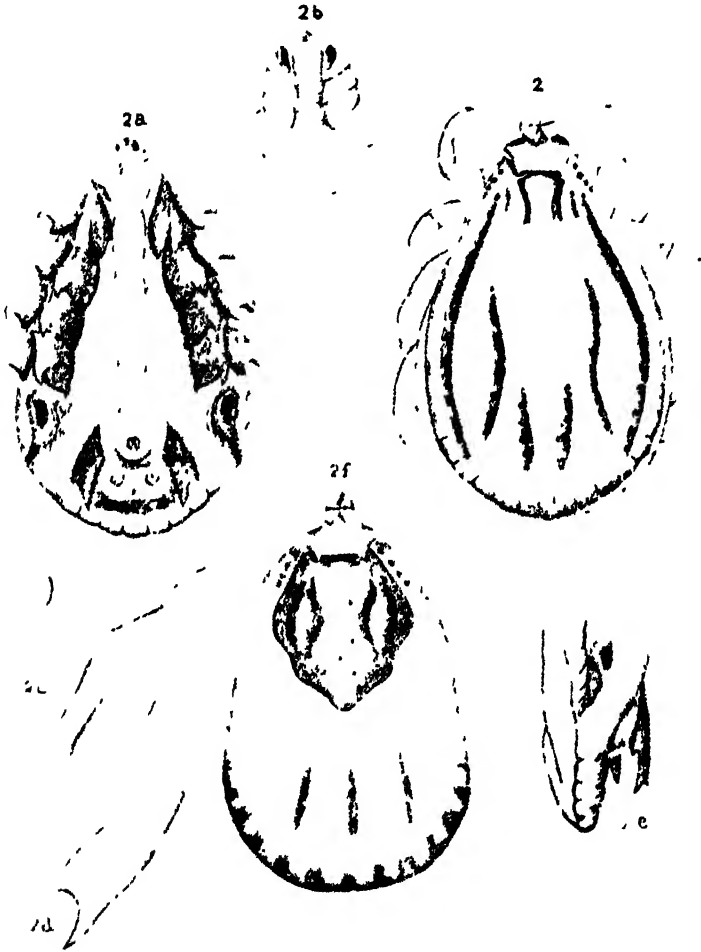


Fig. 1.—*R. armatus*, after Pocock, 1900.

Rostrum.—*Basis capituli* broader than long, lateral angles very salient, in anterior one third. Cornua salient. Two large punctations symmetrical, near straight posterior margin. *Palps* almost as broad as long, flattened dorsally. Articles 2 and 3 the same length, convex externally.

Legs.—Deep brown, strong, stout, with segments punctate.

Ventral Surface.—Anal plates, according to Neumann, punctate, furnished with scattered hairs; triangular, internal margin slightly concave; external shorter and slightly convex; the posterior concave and forming with the internal a long point. Immediately internal to this sharp point a very small triangular plate or spur. Behind the anus and between the anal plates, two small oval plates or sclerites, symmetrical, each situate on a small papilla. No caudal process.

Female (Fig. 2).

Scutum.—"Reddish in middle, blackish at sides", according to Pocock; Neumann gives it as "brownish-reddish"; hardly longer than broad. Eyes in posterior $2/5$; postero-lateral margin sinuous. Cervical grooves deep, stopping at level of the eyes. Lateral grooves deep, formed by large punctations. Punctations according to Pocock, "some large punctations along the lateral edge in front of the eye, a few between the cervical grooves, and a few large ones and many smaller ones on the middle of the posterior area". According to Neumann "about a dozen punctations in the median field; some more, larger, also about one dozen on each pre-ocular field."

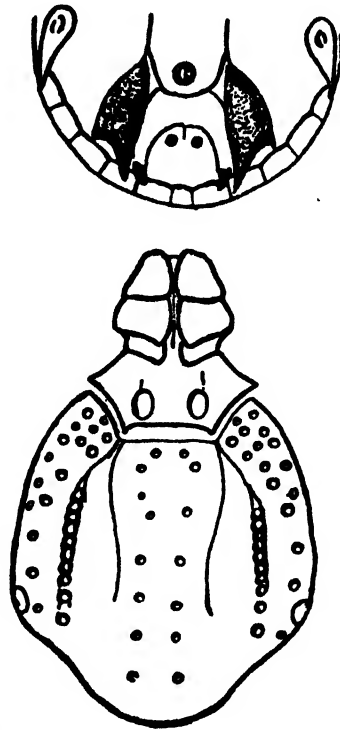


Fig. 2.—*R. armatus*. Top, ♂ ventral view; bottom, ♀ dorsal view, after Neumann, 1901.

Rostrum.—As in the male, with *basis capituli* shorter. Porose areas deep, oval, parallel.

Type.—Collected by Peel 1895 at Bularli, West Somaliland, deposited 1♂, 2♀ in British Museum; 2♂ 1♀ in Hope Museum, Oxford. Neumann's specimens 10♂, 4♀ were collected by Schillings off a lion, British East Africa; deposited in the Berlin museum.

Comments.—Warburton 1912 points out that there are certain rhipicephalids—of which but few examples have ever been found, but which are so peculiar that their claim to specific rank cannot be denied; amongst these he lists *R. armatus*.

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NEUMANN (1901). Revision de la famille des Ixodidae. *Mem. Soc. Zool., France*, Vol. XIV, p. 271, fig. 3.

WARBURTON (1912). *Parasitology*, Vol. V, p. 5.

" RHIPICEPHALUS AURANTIACUS " Neumann 1907.

Male. (Fig. 3.)

4.8 × 2.7 mm.; Coxa I not visible dorsally. *Consutum* slightly convex, shiny reddish brown. Eyes large, flat, light coloured, marginal. Cervical grooves superficial, diffuse; lateral grooves obsolete or superficial, wide, weakly defined along internal edge, short, commencing halfway between eyes and stigmata, including the last festoon. Punctations numerous, fine sub-equal, shallow, non-confluent, slightly closer together in the lateral grooves, or in the region corresponding to the lateral grooves in those cases where they are obsolete; absent in the other depression. Posterior median groove large reaching to level of anus; the laterals shorter and shallower.

Rostrum 0.8 mm. *Basis capituli* twice as broad as long; lateral and posterior angles very salient. *Palps* almost as broad as long, flattened dorsally; Articles II and III about equally long.

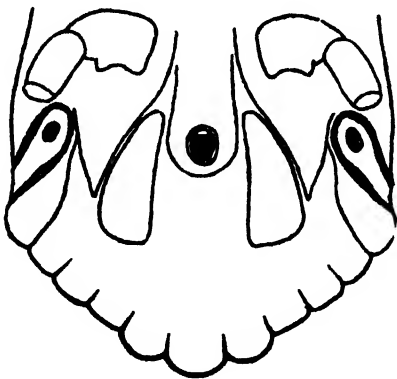


Fig. 3.

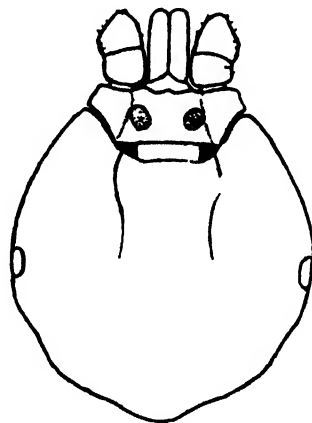


Fig. 4.

Fig. 3.—*R. aurantiacus* ♂, ventral view, after Neumann, 1907.

Fig. 4.—*R. aurantiacus* ♀, dorsal view, after Neumann, 1907.

Ventral surface yellow anteriorly, shading to orange posteriorly. [This orange colouring of the abdomen, which gives the tick its specific name, I have also seen in a batch of *R. evertsi* collected in Namaqualand]. *Anal plates* an inverted comma, internal margin slightly concave, external convex, posterior convex continuing the curve of the external margin and making an angle with the internal. Accessory plates hardly, or not at all, chitinised at their tips.

Female. (Fig. 4).

Body short oval, 5.1 mm. \times 3.25 mm. *Scutum* as wide as long, 2 mm.; postero-lateral margin slightly sinuous. Eyes in middle of the length. Cervical groove shallow at origin, after that wider and very superficial, visible till posterior $\frac{1}{3}$. No lateral groove. Punctations resemble those of male, regularly distributed over entire surface.

Rostrum: *Basis capituli* at least twice as broad as long; lateral angles salient; cornua not prominent. Porose areas shallow, sub-circular; distance apart equal to their diameter, edged externally by a dorsal ridge. *Palps* longer than broad, otherwise as in the male.

Type.—5♂, 3♀ collected by Büttikofer off *Buffelus pumilus*, Iäberia; deposited in the Museum at Leyden.

Geographical distribution.—Also reported by Bequaert 1931 off *Syncerus nana* and off wild pig from Congo Belge and from Liberia; and by Fiasson 1943 off a buffalo and off a *Potamochoerus* from Dolisie, Moyen-Congo.

LITERATURE.

NEUMANN (1907). Quatre espèces nouvelles d'Ixodides. Note XI. Notes from the the Leyden Museum, Vol. XXIX, p. 91, figs.

“ RHIPICEPHALUS COMPLANATUS ” Neumann 1910.

Synonym: *Rhipicephalus planus* Neumann 1910
nec *R. simus planus* Neumann 1907.

Male. (Figs. 5–6.)

A large tick; 4 to 5 mm. by 2.6 to 3.5 mm.; oval in shape, widest at the level of the stigma; inornate.

Conscutum: flat or slightly concave; chestnut brown, lighter in the centre; no ornamentations, slightly shiny. Eyes very flat, medium-sized, yellowish and marginal in position. Emargination as in figure. Cervical grooves short, narrow, deep; lateral grooves shallow, wide, including the penultimate festoon, commencing a short distance behind the eyes; pitted by a few unequal punctations. Punctations scattered, subequal, small, superficial, practically absent in the posterior third in the larger specimens and almost completely absent in the smaller specimens. Festoons sharply marked off from one another, increasing in length from the outermost towards the median, the median is also the largest. No posterior grooves [a posterior median groove figured in the drawing].

Rostrum: 0.85 mm. to 0.95 mm. *Basis capituli* twice as broad as long with three to five punctations; lateral angles not very pronounced, in the

anterior third; posterior cornua strong, but not prominent. *Palps* conform to type; article I visible dorsally; articles II and III equal, internal margin longer than external; twice as broad as long.

Legs: stout, long, dark chestnut brown: Coxa I slightly visible dorsally [only just visible in figure].

Ventral surface: uneven yellowish-brown. Anus towards the anterior quarter of the anal plates. *Anal plates* curved, broad anteriorly, broader still posteriorly; external margin very convex; internal margin forming an obtuse angle opposite the anus, then very concave, then straight and forming a sharp angle at its junction with the posterior margin; posterior margin somewhat concave towards the middle and forming an obtuse angle at either end.

Accessory and plates forming a strong chitinous point.

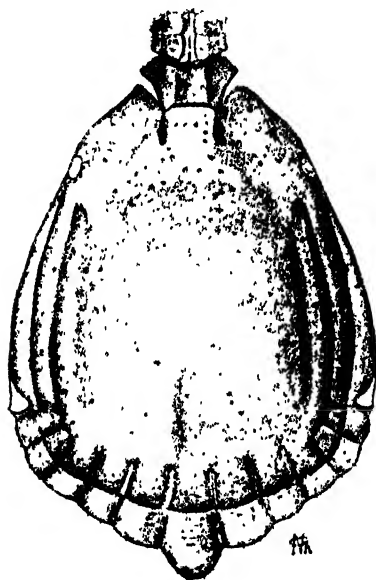


Fig. 5.

Fig. 5.—*R. complanatus* ♂, dorsal view, after Neumann, 1910.

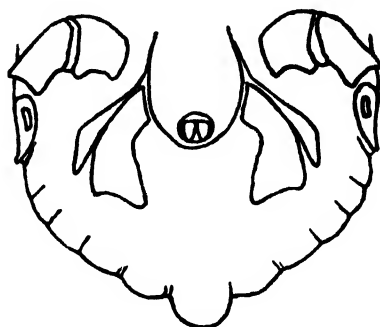


Fig. 6.

Fig. 6.—*R. complanatus* ♂, ventral view, after Neumann, 1910.

Female. (Fig. none.)

5 mm. × 3 mm., oval. *Scutum* wider 1.75 mm. than long 1.50 mm., dark chestnut brown. Eyes as in the male; emargination wide and deep; posterior lateral margin but slightly sinuous. Cervical grooves deep at first, then very superficial; lateral grooves well marked reaching to posterior border. Punctations discrete, small, slightly larger in the median and the lateral fields.

Rostrum: 1.05 mm. long. *Basis capituli*: as in the male; areae porosae small, slightly oval, hardly longer than broad; parallel; the distance apart equal to their greatest diameter. *Palps* longer than in the male; article 1 more visible than in the male.

Type: described from 6 males and one female collected in 1907 by Dr. Gravot, from a dead wild boar, in the Ivindo basin in the South Cameroons and deposited in the Paris Museum.

Geographical distribution.—Also reported by Fiasson, 1943 from *Potamochoerus* off Komono in the Moyen Congo.

LITERATURE REFERENCES.

- R. FIASSON (1943). Contribution à l'étude des Arthropods vulnérants du Moyen-Congo. *Rev. des Sc. Méd. Pharm. & Vet. de l'Afrique Fr. Libre*, Vol. II, No. 3, p. 261.
- NEUMANN (1910). Sur quelques espèces d'ixodidae. *Annales Sc. Nat.*, Ser. IX, Vol. XII, p. 165-168.

RHIPICEPHALUS CUSPIDATUS, Neumann 1906.

Male. (Fig. 7.)

4.7 mm. \times 3 mm. widest in posterior $\frac{1}{4}$, almost as broad anteriorly. Coxa I visible dorsally. *Consutum* slightly convex, dark brown. Eyes flat, yellow, large. Cervical grooves deep and very short; no lateral grooves, festoons longer than broad deeply separated; punctations large, equal, rare, far apart, the majority occupying the place of the lateral margins.

Rostrum: 0.95 mm. *Basis capituli*: broader than long, smooth; lateral angle in anterior $\frac{1}{3}$, very salient; cornua salient. *Palps* hardly longer than broad, flattened dorsally; Article 3 as long as Article 2, article 2 projects posteriorly.

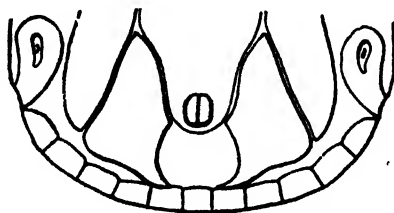


Fig. 7.—*R. cuspidatus* ♂, ventral view, after Neumann, 1906.

Legs.—Stout. *Ventral Surface*: Anal shield quadrilateral, longer than broad; external margin straight; antero-internal margin convex; postero-internal concave, the posterior margin sinuous, forming with the postero-internal a long strong point. Accessory shield hardly chitinous, with numerous hairs. No caudal process.

Female. (No Figure.)

6 mm. \times 4 mm. *Scutum*: Chestnut brown, slightly broader than long 2.43 mm. \times 2.56 mm., margins slightly sinuous; eyes slightly in front of middle of scutum. Cervical grooves deepest at point of origin, wide and superficial, later reaching almost to posterior border. No lateral grooves.

Punctations about one dozen on either side, large, forming a line of 3-4 beyond the cervical groove; one or two in the median field, the rest along the lateral border in front of the eyes.

Rostrum.—*Basis capituli*: Twice as broad as long, with the same outline as in the male. Porose areas deep, oval, separated by about twice their diameter. *Palps*: as in the male, but slightly longer.

Type.—1♂ and 4♀ collected off *Phacochocrus* in Senegal, deposited in the British Museum.

LITERATURE.

Neumann (1906), Notes sur les Ixodidés IV. *Archives de Parasitologie* X p. 209, fig. 11.

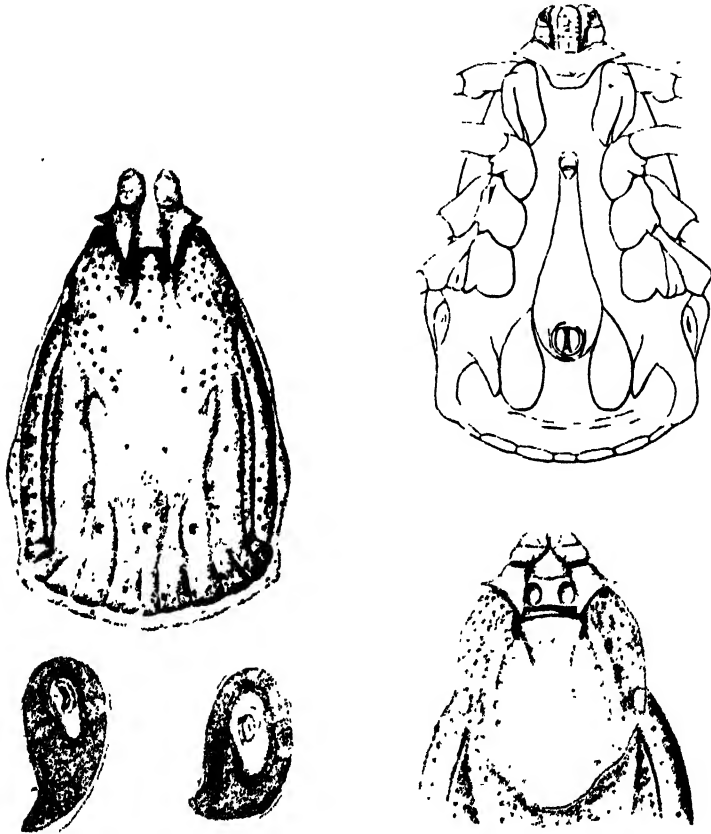


Fig. 8.—*R. deltoideus*. ♂ and ♀, after Neumann, 1910.

RHIPICEPHALUS DELTOIDEUS, Neumann 1910.

Male. (Fig. 8.)

Contour subtriangular, widest behind the middle.

Consutum.—Eyes flat, surface shiny, uniformly brown. Lateral grooves deep, long, commencing near the eye. Posterior grooves present. Punctations fairly numerous; with fine punctations evenly distributed, and large punctations rare and far apart; three or four smooth longitudinal folds.

Ventral Surface.—Anal plate subtriangular with convex posterior margin.

Legs.—Coxa I not visible dorsally.

Female. (Fig. 8.)

Scutum.—Uniformly brown, oval, longer than broad. The cervical field is sunken. Lateral groove well developed. Punctations very uneven, very fine and very large, far apart.

Rostrum.—*Basis capituli*: Width equals three quarters that of the scutum. Porose areas large, distance apart equal to their longest diameter. *Palps* short.

Type. ♂ and ♀ from Basutoland.

The original description is not available to me; the above diagnostic features are taken from Bequaert's 1931 Key. The figures are from the original article.

Occurrence.—Bequaert records it from wild hares, Ishasa River to the North of Rutshuru in the Belgian Congo. The Onderstepoort collection contains specimens which have been provisionally identified as *R. deltoideus*? off a wild hare from near Richmond, Cape; off *Gazella granti brighti* from Lolito, Kasamoja, Uganda; off *Struthio camelius molydophares* from near Kodide, Jie, Karemoja; and off *Neotis caffra jacksoni* from Unyama river, Gulu, Uganda. The Uganda material was all collected by T. W. Chorley and donated by G. H. E. Hopkins.

RHIPICEPHALUS DISTINCTUS, Bedford 1929.

Synonym: *R. punctatus* Bedford 1929.

nec. *R. ncavei-punctatus* Warburton 1912.

Male. (Fig. 9.)

2.26 mm. × 1.5 mm. oval, nearly twice as wide posteriorly as anteriorly. Coxa I visible dorsally. *Consutum* reddish to light brown in colour; shiny. Emargination deep; eyes flat, yellowish, marginal. Cervical grooves very short. Lateral grooves deep, picked out with large punctations, extending to the first festoon, preceded anteriorly by a row of five to six punctations more internal in position. Posterior grooves median, indicated by a fine line, the laterals by a slight dimple, or the posterior grooves may be obsolete. Festoons well developed. Punctations, very few, large, widely spaced, those in the posterior half being particularly large and deep, a few clustered on the shoulder and a few posteriorly; an odd punctation present on one or other festoon; arising from each punctation there is a minute (caducent) pale hair.

Rostrum.—*Basis capituli*: Wider than long, 0.43 mm. × 0.5 mm. Lateral angle very far forward, prominent; posterior margin very slightly concave without pronounced cornua; two or more large punctations. *Palps*: longer than broad. Articles 2 and 3 wider than long, almost equally long. Article 1 visible dorsally.

Ventral Surface.—Reddish brown, with a few punctations, each with a small pale hair. No caudal process. Anal plate elongate, sides almost parallel, internal margin with a very slight concavity, longer than external,

so that the convex posterior margin meets the inner margin at an angle less than a right angle and the outer margin at an angle greater than a right angle; both angles rounded off. Accessory plates: only the tip of the pronounced fold is chitinized, into a sharp point.

Female. (Fig. 10.)

When unengorged the scutum covers over half the body length.

Scutum slightly wider than long, 1.26 mm. \times 1.36 mm., or as long as broad; dark reddish brown, shiny. Emargination wide and shallow. Eyes flat, yellowish. Cervical grooves very short, bent inwards, followed by a slight cervical depression. Lateral grooves entirely absent. Punctations four to five large punctations limiting the raised lateral border (i.e. in the position of a lateral groove); four to five widely separated in the posterior median field; two to four anteriorly on the central field, a few smaller ones clustered on each shoulder. The general scutal surface covered by fine shallow punctations, not seen in the male.

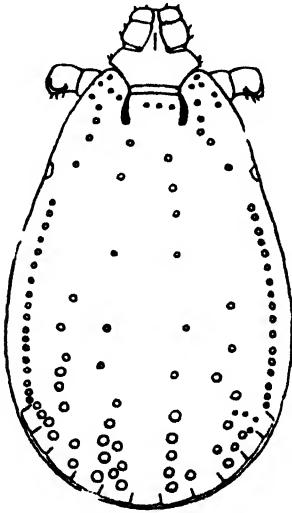


Fig. 9.

Fig. 9.—*R. distinctus* ♂, dorsal view, after Bedford, 1929.

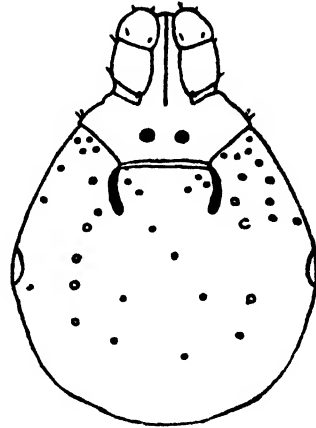


Fig. 10.

Fig. 10.—*R. distinctus* ♀, dorsal view, after Bedford, 1929.

Rostrum.—*Basis capituli*: more than twice as broad as long, lateral angle sharp, far forward, cornua not pronounced. Porose areas rounded, distance apart a little more than their diameter. *Palps.* Articles 2 and 3 about as broad as long, equal in length; Article 1 about three quarter as long as 2.

Nymph. (Fig. 11.)

Closely resembles the female. *Scutum*: 0.48 mm. \times 0.52 mm., slightly broader than long; antero-lateral margin straight, postero-lateral and posterior margin smoothly rounded. Emargination very wide and shallow. Eyes large and conspicuous. Cervical groove bending inwards at first and then diverging to approach the posterior margin; lateral groove straight; edging a well pronounced raised lateral border reaching to posterior margin, forming with the cervical a narrow cervical field.

Rostrum.—*Basis capituli* has the same foreshortened appearance as that of the female, at least three times as broad as long; lateral angle: prominent, forward; cornua weak; postero-lateral margin but slightly concave. *Palps*:. longer than broad, articles 2 and 3 about as broad as long, about equally long. Article 1 visible dorsally.

Type.—Off *Procapra capensis coombsi* from Onderstepoort, deposited in the Onderstepoort collection. The nymph is described from the Kalkfeld material listed below.

Occurrence.—The Onderstepoort collection contains batches off the Dassie (i.e. *Procapra*) from Omaruru, South West Africa; 2 lots off *Procapra capensis* from Leeuwkoppie, Hout Bay, near Cape Town; 2 lots off *Heterohydrax welwitschi volkmanni* from Kalkfeld and from Kamanjab, S.W.A. collected by the Barlow-Transvaal museum expedition; one batch off *Procapra johnstoni matscheie* from Mwanza, Tanganyika; one batch off sheep, Victoria West, Cape Colony.

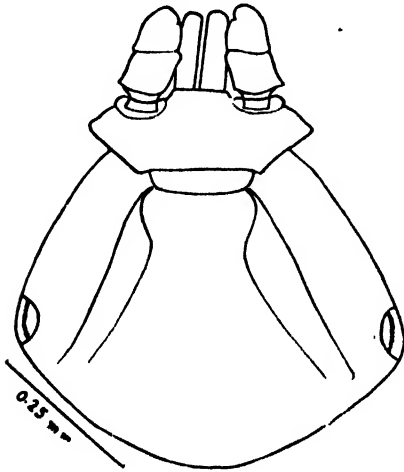


Fig. 11.

Fig. 11.—*R. distinctus* nymph, dorsal view. D. Pringle, del.

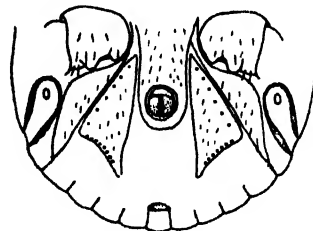


Fig. 12.

Fig. 12.—*R. duttoni*, Rostrum and ventral view, after Neumann, 1907.

The Dassie would thus seem to be its normal host and its distribution is possibly that of its host. Generally speaking, however, Dassies are remarkably free of ecto-parasites, hence the few records, as yet, of *R. distinctus*.

LITERATURE REFERENCES.

- BEDFORD (1912). Notes on some South African ticks with descriptions of three new species. 15th Ann. Rep. Dir. Vet. Serv., Un. of S.Af., p. 495, figs.
 BEDFORD (1932). A synoptic check list and host list of S.A. Ectoparasites. 18th Rep. Dir. Vet. Ser. and Anl. Ind., p. 523.

RHIPICEPHALUS DUTTONI Neumann 1907.

Male. (Fig. 12.)

* 3.5 mm. × 1.85 mm. Narrow in front, broadest a little posteriorly to the middle. *Conscutum*: slightly convex, chestnut brown. Eye flat,

yellowish, marginal; cervical grooves: very broad and shallow and form elongated depressions; they are not punctated and are continued posteriorly by a narrow superficial groove which extends beyond the middle point of the length; lateral grooves: broad, shallow, slightly and finely punctated, commencing immediately behind the eyes and including the last festoon. Posterior grooves: wide, shallow, unpunctated, the median the longest, festoons longer than broad, slightly punctated. Punctations irregular, coarsest in front, fine and superficial over the remainder of the surface.

Rostrum 0.6 mm. *Basis capituli* almost twice as broad (0.6 mm.) as long. Lateral angles: quite prominent at about the middle of the length; cornua: quite prominent. *Palps*: as broad as long; article 2 scarcely longer than article 3, and retracted into a blunt point dorsally at its posterior border.

Legs.—Relatively strong; Coxa I visible on dorsal surface.

Ventral Surface.—Covered by rather long and abundant whitish hairs. *Anal plates*: in the shape of a scaline triangle forming a long internal posterior spine; the internal margin is longest, rectilinear in its anterior half but is concave behind; external margin slightly convex; posterior margin concave and bordered by punctations. The accessory shields are replaced by a prominent non-chitinous fold. Caudal process present.

Type.—1♂ off a bovine at Zambie, Belgian Congo.

Occurrence.—Howard 1908 records a few specimens from the Northern Transvaal; also from Mozambique.

Comments.—Zumpt 1942 recognized this as a distinct species in his *appendiculatus* group; a group characterized by having the inner angles of the anal plates pointed and the accessory anals absent.

To my mind this species is indistinguishable from *R. appendiculatus*, with its third and fourth legs stouter than the first two, and frequently with the anal plate having its internal margin very elongate forming a very definite spine. However, I am hesitant to sink it as a separate species until the type specimen has been re-examined.

RHIPICEPHALUS DUX Dönitz 1910.

Synonym. *R. schwetzi* Larrousse 1927.

Male. (Figs. 13a, 13b.)

A large rhipicephalid; fully engorged, 6 mm. × 3.9 mm.; broadest slightly behind the eye, 3.4 mm. at eye level; compact oval in shape. Ornate, dark brown with white centre.

Consutum.—5.1 mm. in length; eyes quite flat; emargination as in figure; cervical pit small but deep followed by a short groove; lateral groove: indicated by a row of fairly close punctations, enclosing the last festoon; festoons as in figure. Punctations: a few larger punctations present next to the lateral groove as also on either side of the elongate median groove (depression); these larger punctations are also present on the scapulae and on the anterior midregion, and are more irregularly distributed and smaller than in *R. simus*; the smaller, fairly regularly distributed punctations on the other hand are markedly larger than in *R. simus*; the marginal field and the

festoons appear very smooth with only fine punctations; the larger punctations as seen on the 1st festoon on the left in the figure are an exception. The posterior grooves shallow, median groove pointed, the lateral grooves almost circular; these grooves do not join with the festoons. The ground colour is reddish-brown, the middle of the shield is lighter and posteriorly practically white. This lighter area ends abruptly at the level of the posterior edge of the lateral grooves. The colour of the median field becomes darker and browner from the middle of the body forwards; this change is most marked just behind the emargination. The foveolae are immediately in front of the pointed median groove; in front of these are two larger, almost circular and darkened depressions, further apart from one another than the foveolae; the nature of these depressions is not clear.



Fig. 13 (a).

Fig. 13 (a).—*R. dux*, ♂ dorsal view, after Dönitz, 1910.

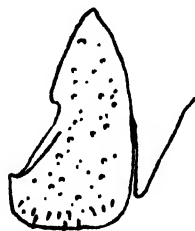


Fig. 13 (b).

Fig. 13 (b).—*R. dux* ♂ anal shield, after Dönitz, 1910.

Rostrum.—*Basis capituli*: twice as broad as long; the cornua not included; 1 mm. \times 0.5 mm. The antero-lateral margin is slightly longer than half of the postero-lateral margins. *Palps* as in figure. The ventral basal plate of article I is very broad.

Palps as in figure. The ventral basal plate of article I is very broad.

Legs: *Coxae* [According to the figure coxa I visible when viewed dorsally.]

Ventral surface: *Anal plates* show the closest resemblance to those of *R. bursa*; they are coarser, with fine punctations (see figure) [accessory anals, as judged by the figure, are apparently represented by a thickened fold of the chitin].

Female. (Fig. 14.)

Large, ornate with lighter median field.

Scutum: 2 mm. long and slightly broader than long. Eyes flat. Lateral groove very pronounced, punctations coarser than in male; grooves and punctations much the same as in *R. simus*, the fine punctations, however, are coarser and the large punctations smaller than in *R. simus*. The entire median field, up to the posterior border lighter in colour, the last third more definitely white.

Dorsum: White scales present in the marginal groove and in the large punctations. These, however, are smaller than in *R. simus*. Ornamentations, such as are seen in *R. pulchellus* and *R. maculatus*, absent.

Rostrum: *Basis capituli* twice as broad as long. *Areae porosae* fairly large and their own diameter apart.

Affinities (Dönitz 1910): differs from the other two ornate ticks, *R. pulchellus* and *R. maculatus*, in having a broad *basis capituli*; and in the male *R. dur* the posterior grooves are well developed, whereas in the other two species they are absent.

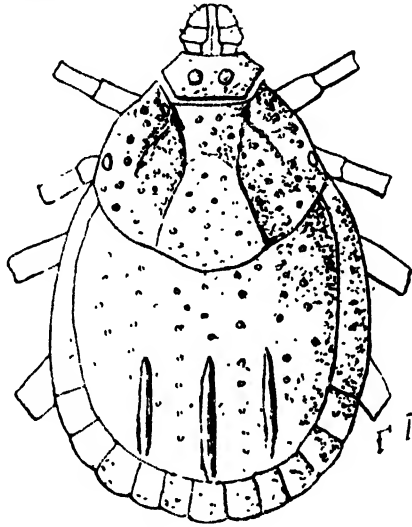


Fig. 14.—*R. dur*. ♀, after Larrouse, 1927.

Type: 1 male and 1 female from the Upper Congo.

Host: Probably the elephant in that the one specimen was included in a container with two *Amblyomma tholloni* and this latter species has thus far only been recorded from the elephant.

Distribution: recorded as *R. schwetzi* off *Hylochoerus itiuriensis* at Kotele, Belgian Congo; Bequaert (1931) reports it off *Syncerus planiceros*, the forest buffalo, at Medje, and off *Potamochoerus porcus* at Avakubi, Belgian Congo.

LITERATURE REFERENCES.

- BEQUAERT (1931). *Rev. Zool. Bot. Afr.*, Vol XX, No. 3, p. 337.
 DÖNITZ, W. (1910). Zwei neue Afrikanische Rhipicephalusarten (*R. dur* *R. glyphis*). *Sitzungsberichte Ges. Naturf. Freunde, Berlin*, No. 6, 1910.
 LARROUSSE (1927). (*R. Schwetzi*). *Rev. Zool. Afr.*, Vol. XV, p. 214-216, figs.

" RHIPICEPHALUS FALCATUS " Neumann 1908.

Male. (Fig. 15.)

With rostrum 4·3 mm. to 4·8 mm. long by 2·7 to 3 mm. broad, at the level of the stigma; inornate; dark brown.

Conscutum: Slightly convex, not shiny, dark brown, smooth. Eyes medium, flat; yellowish. Emargination as in figure. Cervical groove very short, followed by a narrow depression. Lateral groove pronounced, beginning a short distance behind the eye and forming a continuation of a line further in, interrupted by large punctations; including the penultimate festoon. Festoons pronounced. Punctations: numerous, medium occupying the entire field between the lateral grooves, larger and closer together in the posterior third; practically absent on the festoons and on the marginal field. No posterior grooves (present, but faint, in the Onderstepoort specimens).

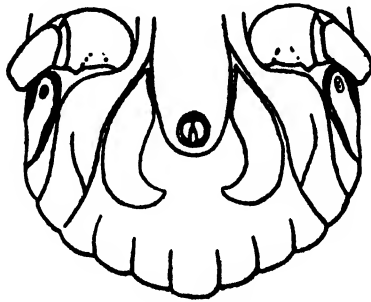


Fig. 15.—*R. falcatus* ♂. dorsal view, after Neumann, 1908.

Rostrum: 0·8 mm. to 0·95 mm. in length. *Basis capituli* broader than long 3:2; lateral angle towards the anterior third; cornua but slightly prominent. *Palps*: slightly longer than broad, flattened dorsally; Article II and Article III about the same length; posterior margin of article II tapering to a large point, and some distance from the basis capituli (i.e. article I partly visible dorsally).

Legs stout. *Coxa I* not visible or but slightly visible dorsally.

Ventral surface: Yellowish-white, glabrous; anus towards the middle of the anal plates. Anal plates sickle-shaped; the internal margin very concave posteriorly, forming a point with the posterior margin. Posterior margin forming a continuous convex curve with the external margin; accessory anals hardly or not at all chitinated. Festoons pronounced, with a dark spot along the free edge; median festoon often more prominent than the rest; the two neighbouring festoons are also enlarged sometimes.

Female.

Body oval, 4 mm. to 6 mm. long by 2 to 3 mm. broad at the level of the stigmata; dark brown.

Scutum: brown, slightly shiny; as broad as long; 2 mm.; posterior margin slightly sinuous. Eyes about halfway, flat, narrow. Cervical grooves not deep at their origin and then broad and very superficial, reaching about halfway back. Lateral grooves pronounced, edged externally by a raised

lateral field reaching to posterior border. *Punctations*: numerous, medium, subequal, absent along the cervical margin, along the posterior margin and practically absent along the preocular margin.

Rostrum: *Basis capituli*: at least twice as broad as long; lateral angles prominent; posterior angles scarcely prominent. *Areae porosae* oval, longer than broad; parallel, distance apart equal to their length. *Palps*: rather longer than broad, otherwise as in the male.

Type: described from 3 males and 8 females collected by Old, North of Lake Nyassa and deposited in the British Museum; and 4 males and 1 female collected by F. X. Stämpfli in Liberia and deposited in the Leyden Museum.

Comments.—Warburton in 1913 remarks, in connection with punctations on the scutum; "There is certainly for each species a characteristic punctation very recognizable in typical examples, but often widely departed from in individuals or in local varieties, and when this is the case the difference in facies between the ticks otherwise structurally identical may be very great. A striking case is *R. falcatus*, a densely punctate form, which at first glance bears no resemblance at all to *R. simus* where the punctations are few and arranged in linear series. Moreover *R. falcatus* typically possesses very characteristic anal plates quite unlike those we are accustomed to expect in *R. simus*, and there is no anterior prominence on coxa I. Yet we can find no other structural points in which the forms differ, and moreover we possess a tube of ticks from Nyasaland, which we have been quite unable, after repeated attempts, to sort out. There are many undoubted *R. falcatus*, a considerable number of obvious *R. simus* and every intermediate grade of anal plate, prominence of coxa I and punctations."

Warburton (1913) also degrades *R. lunulatus* Neumann 1907 (= *R. tricuspis* Dönitz 1906) to a variety of *R. simus*.

From my experience of South African ticks I find that although anal plates may show some variations within a species, the range is never so great as is assumed here, i.e., so great as to vary from a narrow sickle-shaped plate, through a bean, to kidney-shaped plate, still with convex external and posterior margin, to a plate such as we find in *R. tricuspis*, where the external margin is only slightly convex and where the posterior margin is definitely concave. Hence, since I disagree with the conclusions drawn by Warburton (1913) I am republishing the description of this tick under its original name of *R. falcatus*. A more detailed analysis of *R. simus* and of *R. tricuspis* will be given later, the analysis to be based on the offspring of several individual females.

Distribution.—*R. falcatus* is listed as present North of Lake Nyasa, Neumann 1908; Kenya, Lewis; Liberia, Neumann 1908; the Onderstepoort collection has specimens from a buffalo, Borôr, Portuguese East Africa; and from *Phacochoerus aethiopicus*, Gulu district, Uganda; off buffalo at Mabindi and Hoima, East Africa.

LITERATURE REFERENCES.

- NEUMANN (1908). Notes sur les Ixodidés—XI. Notes from the Leyden Museum, Vol. XXX, p. 77.
- WARBURTON (1912). Notes on the Genus *Rhipicephalus*. *Parasitology* V.

RHIPICEPHALUS FOLLIS Dönitz 1910.*Malc.* (Figs. 16-17.)

A medium-sized, squat tick, brown.

Conscutum: 3·9 mm. to 4·2 mm. \times 2·8 mm. to 3 mm.; convex; broad anteriorly. Eyes 1·9 mm. apart. Cervical groove, see figure; lateral groove well developed, ends at the penultimate festoon. Posterior grooves shallow, deeper in the one specimen than in the other, median groove does not reach the festoons; postero-laterals joined to the fourth festoon by a delicate prolongation; in the one specimen the postero-lateral groove forms a groove, in the other a broad depression which is slightly rugose and shows a few punctations. Punctations are fairly fine, superficial, fairly closely and evenly distributed; larger punctations present as uneven rows in front of the eyes and scattered separately over the scutum. In some places the punctation is denser, in others fine, sharply defined, microscopic punctations are present; but few punctations present on the lateral border.

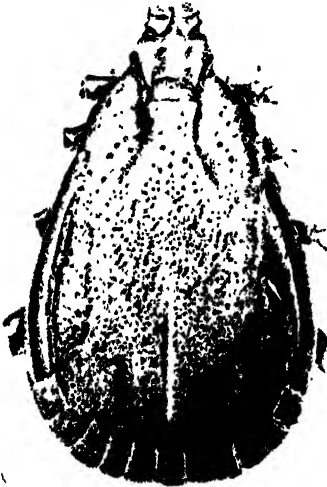


Fig. 16.

Fig. 16.—*R. follis* ♂ dorsal view, after Dönitz, 1910.

Fig. 17.

Fig. 17.—*R. follis* ♂, ventral view, after Dönitz, 1910.

Rostrum: *Basis capituli*: about ·5 mm. long, not twice as broad as long; postero-lateral border about twice as long as the antero-lateral.

Ventral surface: Anal plates very broad posteriorly and resemble those of *R. bursa*; accessory plates well developed. *Festoons*: large, the middle one half as broad again as its neighbours; their posterior margin shows a fairly white edging, which is unknown in any other *Rhipicephalus*; even in the ornamented forms *R. maculatus* and *R. pulchellus* the festoons are uniformly brown.

Legs: Coxa I not visible on the dorsal surface according to the figure

Female. Unknown.

Type.—2 males, origin unknown, probably off domestic stock, South Africa.

Comments.—I have come across a tick off domestic stock from certain parts of the Cape Colony and of the Orange Free State which answers to *R. follis* in every other respect, except that the white band edging the festoons, as given in the diagnostic characters by Dönitz, is absent. If Dönitz had not made the definite comparison with the white enamel-like ornamentation of *R. maculatus* and of *R. pulchellus* one would be inclined to think that what he was describing was not the edge of the scutal festoons, but merely the lighter coloured abdomen which often protrudes beyond the conscutum. His figure of the dorsal surface is not very helpful for he neither shows the white edge to the festoons nor does he show the abdomen protruding beyond the scutum.

If, however, the statement as to the white edging to the festoons is meant to apply to the ventral surface, as may be assumed from its position in the detailed description, then his figure does bear out his detailed description. In the specimens which I have examined, however, I have not seen this lighter band on the ventral surface. The only explanations which I can offer at this stage, without the re-examination of the original material, is that Dönitz was working with dried specimens; in dried specimens, which are full of air, the abdomen sometimes does take on a hard enamel-like appearance.

If the surmise, that Dönitz was working with dried specimens, is correct, then the ticks which I have examined can be taken to be *R. follis*, and this would confirm Dönitz' supposition that the normal hosts are domestic stock, mostly cattle, and that *R. follis* occurs in South Africa.

LITERATURE REFERENCE.

DÖNITZ (1910). Die Zecken Süd Afrika's. L. Schultze Forschungsreise. Bd. IV, Teil. 16. Jen: *Denkschriften*, Bd. XVI.

“ RHIPICEPHALUS JEANNELI ” Neumann 1913.

Male. (Fig. 18.)

5 mm. × 2.7 mm. widest at stigma; oval in shape, narrowing anteriorly; sides convex, posterior margin rounded. Anterior process of Coxa I slightly visible dorsally.

Conscutum: inornate, dark chestnut brown, curved, shiny. Eyes: flat, medium sized, marginal, slightly coloured. Cervical groove: large, very superficial, otherwise almost reduced to a deep anterior pit. Lateral grooves: shallow, narrow, picked out by punctations, beginning a short distance behind the eyes. Festoons well marked; the last one slightly bigger than the others and slightly broader than long; the others longer than broad, the median the largest. No caudal appendage. Dorsal grooves absent, or but merely indicated. Punctations, numerous, fine, unequal; the majority very fine; may be confluent in front of the festoons; scarce on the lateral border and on the festoons.

Rostrum: dark, 0.85 mm. *Basis capituli* broader than long, 0.8×0.56 mm.; lateral angles towards anterior third, only slightly salient; cornua short narrow subacute, a few punctations in the middle. *Palps*: about as broad as long; articles 2 and 3 about equally long; article I relatively long.

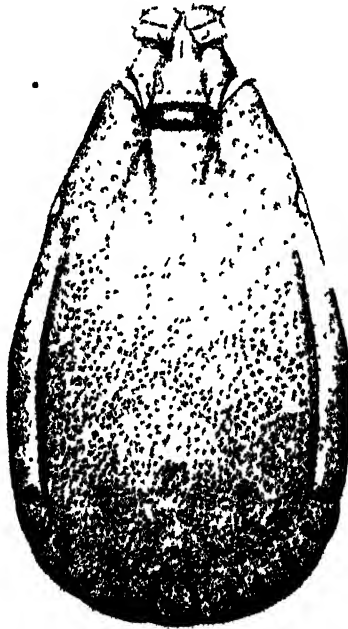


Fig. 18 — *I. jeannelli* ♂, dorsal view, after Neumann, 1913

Legs: relatively strong; coxa I visible dorsally: *Ventral surface*: dark, with numerous hairs. *Anal plates*: subtriangular; anterior and pointed; internal margin concave opposite the anus, external margin hardly convex; posterior forms a wide curve with a small point at its junction with the internal margin; smooth, hardly punctate. Accessory anals chitinous, dark.

Female. (Fig. 19.)

4.1 mm. \times 2 mm. unengorged; widest about midway, oval, narrowing anteriorly.

Scutum: almost as long as broad, 1.7 mm. Eyes pale, slightly behind midway; postero-lateral margin slightly sinuous. Cervical margins slightly more pronounced than in the male; lateral grooves absent or hardly indicated. Punctations numerous, slightly larger than in the male.

Rostrum: 0.81 mm. in length. *Basis capituli* twice as broad (0.76 mm.) as long (0.4 mm.). Lateral angles rather more pronounced and not as far anteriorly as in the male. Cornua short, narrowed and more pointed than in the male. Porose areas, deep, oval; internal equal to their shortest diameter; a dorsal ridge touching their external margin. *Palps* as in the male.

Legs: more slender than in the male. Coxa I not visible dorsally.

Described from—

4 males, 5 females, Mala; British East Africa, 1912.

1 male, 1 female, Lower Regions of Mt. Kenya, 1912.

2 males, Bismarckhügel, Kilimandjaro, German East Africa, 1912.

1 male, Bismarckhügel, Kilimandjaro, German East Africa, 1913.

LITERATURE REFERENCE.

NEUMANN, L. G. Voyage de Ch. Alluaud et R. Jeannel en Afrique orientale 1911-1912. *Resultats Scientifiques Arachnides II Ixodidae*, p. 31-34.

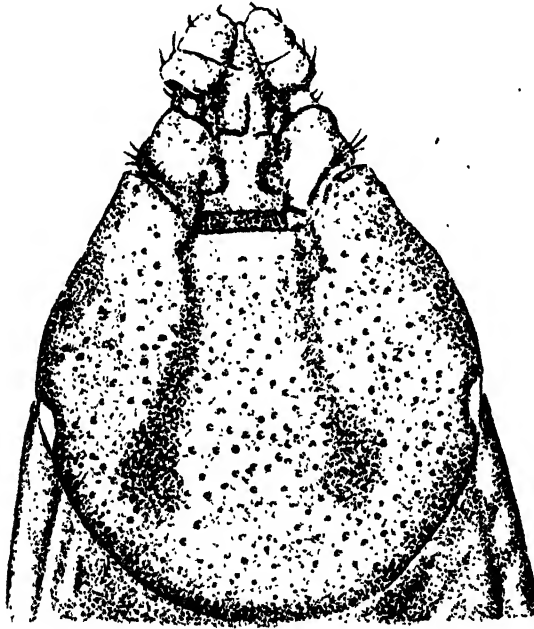


Fig. 19.—*R. feannelli* ♀, dorsal view. copied from Neumann, 1913, by G. E. Laurence.

“*RHIPICEPHALUS KOCHI*” Dönitz 1905.

Male. (Figs. 20-21.)

Size not nearly as broad as *R. ecinctus* (= *R. maculatus*); in shape and size resembles *R. sanguineus* most closely. *Inornate*; Coxa I visible dorsally.

Conscutum: eyes flat, slightly further back than in *R. sanguineus*. *Emargination*: see figure. Cervical grooves short and deep. Lateral groove absent; in its place are to be found large spots, fairly close together arranged in rows (this feature is not shown in Dönitz' drawing). *Festoons* short. *Punctations* equal, slightly smaller than the large punctations of *R. sanguineus*, evenly distributed and close together; along the margins there may

be an admixture of smaller punctations. The three posterior grooves absent; the median is represented by a fine microscopic line; in place of the two postero-lateral, under higher magnifications, a finely chagrined area may be seen.

Rostrum.—*Basis capituli* broader than long, with prominent lateral angles; antero-lateral border only slightly shorter than the postero-lateral border.

Ventral Surface.—Anal-plates very broad, as in *R. bursa*; the external and internal margins almost equally long; internal with a very slight concavity (hardly indicated in Donitz' drawing).

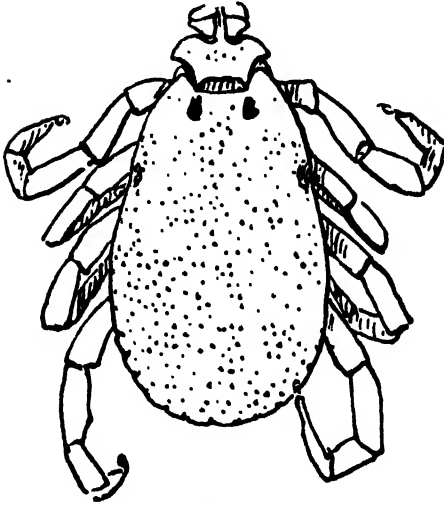


Fig. 20.

Fig. 20.—*R. Kochi* ♂, dorsal view, after Dönitz, 1905.

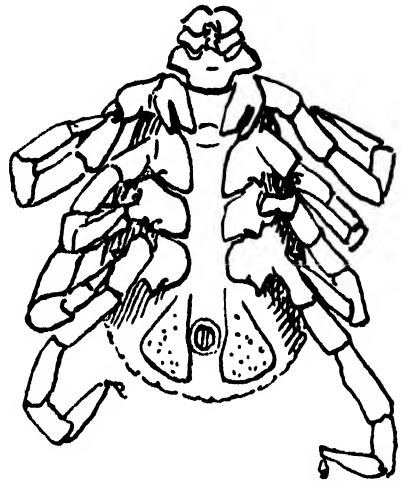


Fig. 21.

Fig. 21.—*R. Kochi* ♂, ventral view, after Dönitz, 1905.

Female. (Fig. 22.)

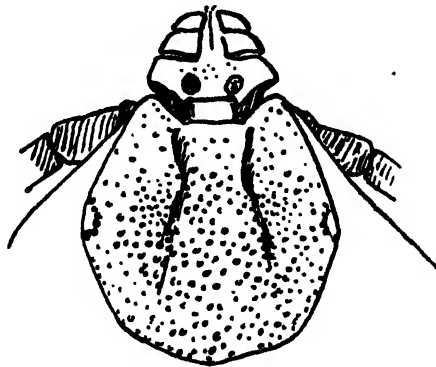


Fig. 22.—*R. Kochi* ♀, dorsal view, after Dönitz, 1905.

Scutum.—Sub-circular, with corners well rounded off; rather longer than broad. Cervical groove as in figure. Lateral groove absent; the raised lateral

field merges gradually with the cervical field. Punctations as in the male, i.e. medium-sized punctations, fairly close together, with a few smaller punctations in the lateral fields.

Rostrum.—Areae porosae more than their diameter apart.

Legs.—Weakly developed, but not quite as slender as in *B.decoloratus*.

Type: described from 1 male and 5 females from Soadani and 3 females from Lindi, East Africa, collected on cattle.

Comments.—In many respects *R. kochi* closely resembles the East African *R. neavei* i.e. in the close even punctations, the "hunched" basis capituli, and the small palps; but differs from it in the absence of the lateral groove, well marked in *neavei*; in the triangular anal plates, in *neavei*, the anal plate has a protuberance on the postero-internal angle and the postero-external angle is almost a right angle; the punctations are evenly distributed, in *neavei* they are absent in the region immediately in front of the eye.

LITERATURE REFERENCE.

DÖNITZ (1905). Die Zecken des Rindes als Krankheits-überträger. *Sitzb. Ges. Natf.* Berlin, 1905.

RHIPICEPHALUS LONGICEPS Warburton 1912.

Male. (Fig. 23.)

Inornate. Anterior projection of coxa I strongly prominent anteriorly. *Conscutum* about 3 mm. \times 1.8 mm. red brown; cervical grooves nearly circular pits, not continued as posterior depressions; lateral grooves well marked, including one festoon. Posterior grooves deep, linear, nearly parallel, subequal. Punctations very numerous, deep uniform discrete, on every portion of the scutum, including the lateral borders and the festoons. Festoons longer than broad and very punctate. Caudal appendage unusually strong, but without a terminal plaque.

Rostrum.—*Basis capituli* of the *R.appendiculatus* type, not much broader than long; lateral angles distinctly anterior and slightly obtuse; the postero-lateral margin about twice as long as the antero-lateral; posterior margin straight, with fairly marked sharp cornua; numerous punctations. Ventral auricular ridges slight. *Palps*: Rather long, flat or slightly concave dorsally, article 3 longer than 2, and with posterior raised edge; Article 1 fairly visible dorsally.

Legs.—Rather long.

Ventral Surface.—Yellowish white in all specimens. *Anal plates* somewhat clavate, usually with an internally directed point (as in *R.capensis*); they tend to become broader distally in large specimens. Accessory plates long, superficial strips of hard chitin, salient posteriorly.

Female. (Fig. 24.)

Scutum.—Sub-circular, deeply emarginate. Cervical grooves fairly deep and only slightly convergent; lateral grooves fairly well marked for two thirds the length; deeply punctate all over. Dorsum with numerous very large punctations.

Rostrum.—Remarkably long, 0.8 mm. *Basis capituli*: punctate, with straight posterior margin and slight cornua; not much broader than long; lateral angles distinctly anterior and slightly obtuse; porose areas large, the interval rather greater than the diameter. *Palps* with article I long, but partly concealed by article 2, which is very long and produced backward to a point; article 3 long and narrowing distally.

Type.—Described from 18♂ and 3♀ from a Klipspringer, collected by Dr. F. Wellman 1907, in the Benguella Hinterland, Angola. Long. E.15° 05'; lat. 12° 44'; altitude 1,360 M; and 19♂ and 2♀ in a mixed collection from the same district.

Types in Cambridge.

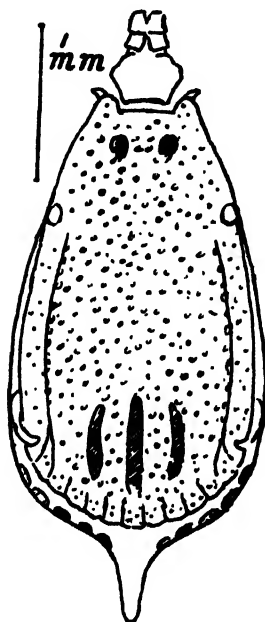


Fig. 23.



Fig. 24.

Fig. 23.—*R. longiceps* ♂, dorsal view, after Warburton, 1912.

Fig. 24.—*R. longiceps* ♀, dorsal view, after Warburton, 1912.

RHIPICEPHALUS LONGICOXATUS Neumann 1904.

Male. (No. fig.)

4.5 mm. × 3 mm. widest towards middle; almost as broad anteriorly as posteriorly. *Consutum*: slightly convex, shiny, reddish brown. Anterior prolongation of coxa 1 visible dorsally. Eyes flat, yellow, large, not quite marginal and at relatively long distance from the anterior end. Cervical groove deep and short. Lateral groove absent. Festoons short, ill-defined. [No mention is made of the posterior grooves.] Punctations large, far apart, in irregular rows at the sides, intermixed with numerous fine punctations.

Rostrum.—*Basis capituli*: broader than long; punctate posteriorly; lateral angles in anterior third very salient; cornua broad and not prominent. *Palps*: slightly longer than broad, flattened dorsally; sides parallel, not extending beyond the hypostome, without lateral projection.

Legs.—Stout, segments fully punctate. Coxa 1 visible dorsally.

Ventral Surface.—No caudal process. Yellowish, reddish with numerous long hairs. Anus at level of anterior third of the anal plates. *Anal plates*: strongly punctate, triangular, internal margin slightly concave; external subrectilinear, the posterior convex and at least half the length of the external. Accessory plates short only slightly chitimized.

Female. (No figs.)

12 mm. \times 8 mm. reddish brown.

Scutum.—Slightly longer than broad, 2.6 mm. \times 2 mm.; postero-lateral margin slightly sinuous. Eyes as in male half way; cervical grooves deep at point of origin, wider and superficial later, hardly extending beyond level of the eyes. Lateral groove absent. Punctations as in the male, i.e. large, far apart, in irregular rows at the sides, intermixed with numerous fine punctations, almost obsolete.

Rostrum.—*Basis capituli* twice as broad as long.

Porose areas deep, oval, separated by twice their length. *Palps* slightly longer than in the male.

Type.—1♂, 2♀, collected by Schillings in German East Africa.

Comments.—This species has not been recorded again. The description, though meagre, would be adequate if accompanied by drawings. The re-examination of the type specimens seems indicated.

LITERATURE REFERENCE.

NEUMANN (1904). Notes sur les Ixodes, Note III. *Arch: Parasit* IX, p. 225, no figs.

RHIPICEPHALUS MASSEYI Nuttall and Warburton 1907.

Synonym: *Rhipicephalus attenuatus* Neumann 1908.

Male. (Figs. 25, 27, 29.)

2.8 mm. \times 1.8 mm. to 4.3 mm. \times 2.6 mm.

Consutum.—Narrow anteriorly, widening behind the level of the eyes, bluntly rounded posteriorly, shiny. Cervical grooves deep crescentic pits, followed by shallow divergent depressions; lateral grooves not well marked, represented anteriorly by punctations. Two pairs of shallow depressions on either side of the posterior median groove. (The Onderstepoort material only shows the usual one pair of postero-lateral grooves). Foveolae visible as small circular pits, far apart between the anterior depressions (indicated by the two black dots on Nuttall and Warburton's figure). Punctations: many shallow, especially on the scapulae, between the cervical grooves, in the marginal grooves, on the festoons and in the posterior region.

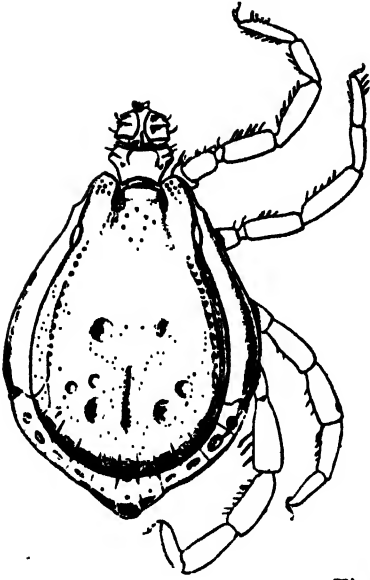


Fig. 25.

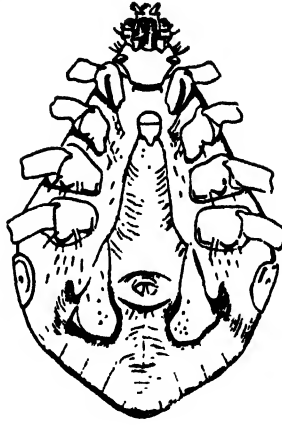


Fig. 26.

Fig. 25.—*R. masseyi* ♂, dorsal and ventral views after Nuttall and Warburton, 1908.

Fig. 26.—*R. masseyi* ♀, after Nuttall and Warburton, 1908.

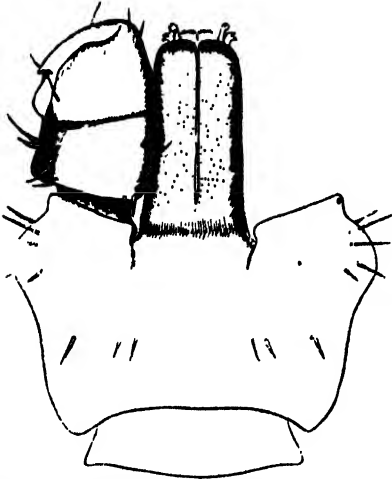


Fig. 27.—*R. masseyi*. Rostrum, dorsal and ventral view, after Nuttall and Warburton, 1908.

Rostrum.—0.7 mm. — 0.8 mm. *Basis capituli* rather long, lateral angle somewhat anterior. *Palps*: Article 1 visible dorsally. Articles 2 and 3 as in figure.

Legs.—Rather long and slender. In the Onderstepoort material the anterior protuberance of coxa I visible dorsally.

Ventral surface.—Hairy. Anal plates roughly isosceles with rounded angles, but sometimes protruding at inner angle. Accessory plates small, bluntly triangular, points. Anus about midway. Caudal process blunt.

Female. (Figs 26-28-30.)

3.6 to 11.6 × 8.9 mm.; somewhat square when full fed.

Scutum.—1.4 mm. to 1.7 mm. × 1.3 mm. to 1.8 mm., in some specimens more elongated. Short oval, shiny; postero-lateral margin convex to sinuous. Eyes large, flat. Cervical grooves deep crescentic pits followed by shallow divergent depressions. No lateral grooves but a raised lateral border. Uniformly punctate, rather fewer punctations on lateral border.

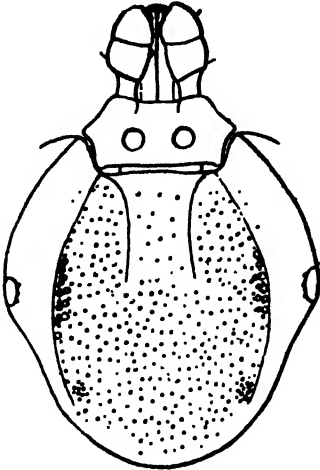


Fig. 28.

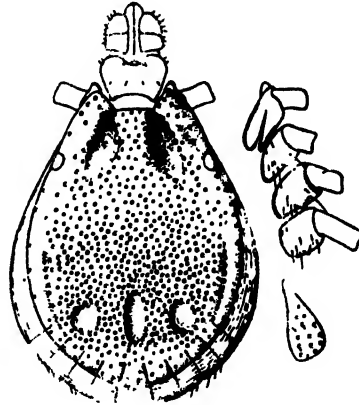


Fig. 29.

Fig. 28.—*R. masseyi*, ♀ dorsal view, after Neumann, 1908.

Fig. 29. *R. masseyi*, ♂ Bedford, del.

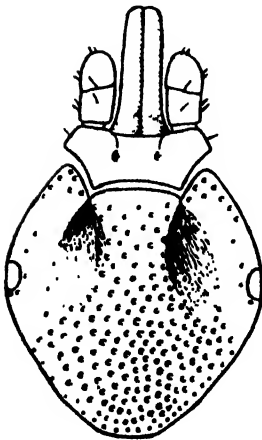


Fig. 30.

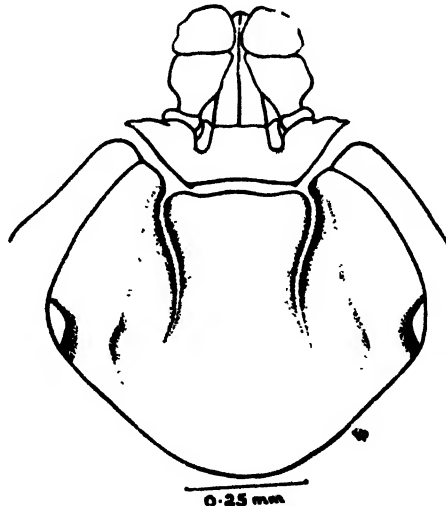


Fig. 31.

Fig. 30.—*R. masseyi* ♀. Bedford, del. Figs. 25-30 show the specific variations very clearly.

Fig. 31.—*R. masseyi*, nymph, dorsal view. D. Pringle, del.

Rostrum.—*Basis capituli*, shorter than in the male with lateral angles more pronounced than in the male. Porose areas oval, medium sized, edged externally by a slight dorsal ridge, which latter terminates in the blunt cornua. *Palps* as in the male.

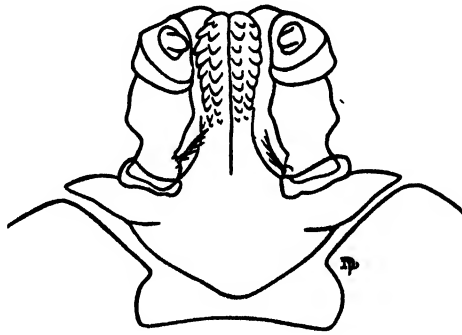
Nymph. (Figs. 31-32.)

As in figure. *Basis capituli* wider than long; lateral angle swings into a very sharp and well pronounced auricula on the ventral side, also seen in the adults i.e. the widest part of the basis capituli is ventrally displaced; very slight cornua present.

Palps.—Article III if anything wider than article II.

Type.—*Attenuatus*: 1 ♀ of *Equus caballus*, Kansanshi.

Masseyi 31♂ and 21♀ collected by Dr. A Yale Massey in 1907 off *Bos caffer* at Kansanshi, N.W. Rhodesia.



0.25 mm.

Fig. 32.—*R. masseyi*, nymph. Rostrum, ventral view. D. Pringle, del.

Geographical distribution.—The Onderstepoort collection contains batches off the Nyala collected on the Ubombo flats, and in the Mkuzi game Reserve in Zululand, and off *Phacochoerus aethiopicus sundevalli* from the Tsetse Research station on the Lower Umfolozi, Zululand.

Discussion.—Neumann's description differs from that of Nuttall and Warburton on one point only, namely according to Neumann's description the lateral grooves are well marked; for the rest the descriptions are identical. In studying the Zululand material it is seen that what Neumann figures and describes, is actually a raised lateral border which rises fairly abruptly from the shallow cervical field. Internally, in some instances, the almost smooth border is lined by a row of widely spaced punctations, without, however, the formation of a definite groove. If Nuttall and Warburton had put into their drawing the external margin of the shallow divergent depressions which lead off from the deep cervical pits, then their figure would agree with their description and with the figure given by Neumann. The punctations on the lateral grooves are never as abundant as on the rest of the scutum as is indicated in both their drawings.

The *Rhipicephalus* sp. of Bedford's 1932 key proves to be *R. masseyi*.

LITERATURE.

- BEDFORD (1932). Key and check list South African Ectoparasites, 18th Rep. Dir. Vet. Ser. and. Anl. Ind.
- NEUMANN (1908). Notes sur les Ixodes VI. *Archiv: de Parasitol.* XII, p. 12, fig. 8.
- NUTTALL AND WARBURTON (1907). On a new genus of Ixodoidea, together with a description of eleven new species of ticks. *Proceedings Camb. Phil. Soc.* XIV, p. 405.

" RHIPICEPHALUS SCULPTUS " Warburton 1912.

Male. (Fig. 33.)

A large tick up to 4 mm.; anterior projection of Coxa I visible, though not prominently so, the projection curving outwards.

Consutum: Lateral grooves and dorsal grooves much the same as in *R. supertritus*; sculpture very characteristic, glossy, raised ridges defining a very distinct pseudo-scutum (female scutum) and outlining the posterior grooves; the rest of the surface consisting of extremely rough shagreened tracts from which arise raised areas which are deeply punctate.

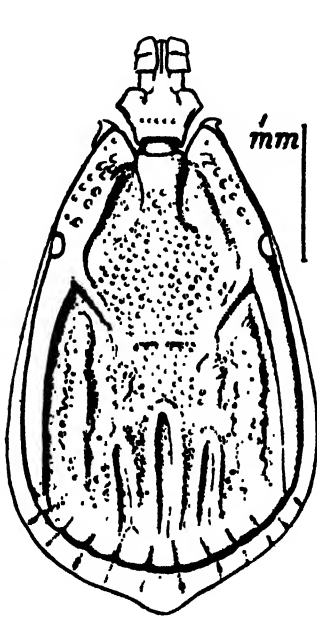


Fig. 33.

Fig. 33.—*R. sculptus* ♂, dorsal view, after Warburton, 1912.

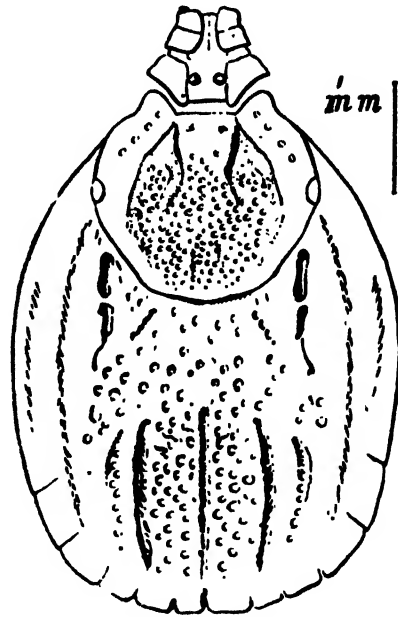


Fig. 34.

Fig. 34.—*R. sculptus* ♀, dorsal view, after Warburton, 1912.

Rostrum: *Basis capituli:* not much broader than long, lateral angles anterior.

Legs: yellowish, contrasting strongly with the dark brown of the consutum.

Ventral surface: Anal plates much as in *R. supertritus*; accessory anals absent.

Female. (Fig 34.)

Like *R. supertritus* but larger.

Scutum 1.8×1.8 mm. Lateral ridges less divergent and longer, converging behind the eyes, so that the whole strongly punctate central area is framed by a glossy, raised border; a raised punctate area or island is present in the region between the cervical grooves and lateral ridges. Dorsum strongly punctate and grooved, with short white hairs, extremely stout and thickest especially along the marginal grooves.

Type: collected from a roan antelope, Mpalali River, Marimba, Nyasaland; from a zebra; S. Rukura Valley, N. Nyasaland; deposited in the British Museum and Cambridge.

Comments: "*R. appendiculatus*, *R. supertritus* and *R. sculptus* are three forms closely allied and in certain structural points practically identical, but presenting a different facies on account of the progressively complicated scutal sculpture in both sexes." Warburton 1912.

LITERATURE.

WARBURTON (1912). Notes on the Genus *Rhipicephalus*. *Parasitology* I, p. 13, figs.

"*RHIPICEPHALUS SIMPSONI*" Nuttall 1910.

Male. (Fig. 35.)

Coxa I showing slightly dorsally.

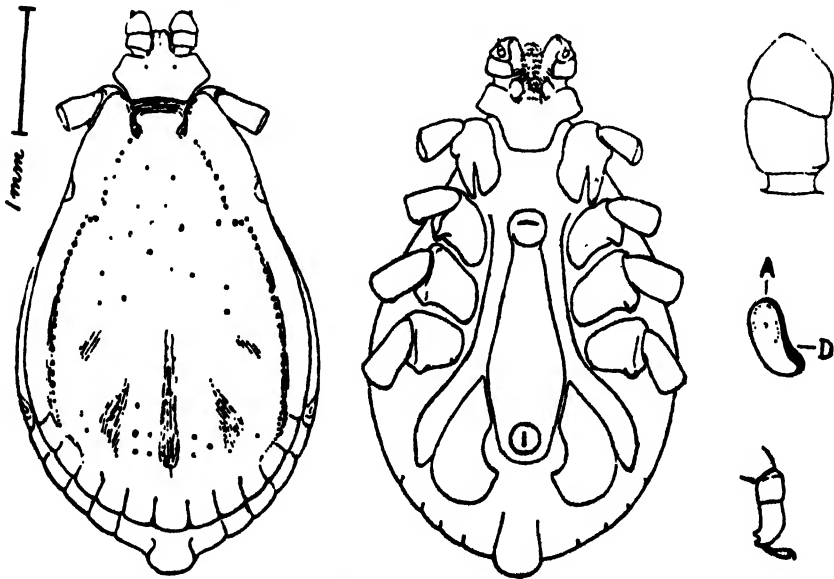


Fig. 35.—*R. simpsoni*, ♂, dorsal and ventral view, after Nuttall, 1910.

Conscutum 2.3 mm. \times 1.5 mm. to 3.6 mm. \times 2.2 mm.; pear-shaped broadest at $\frac{3}{4}$ body length. Deeply emarginate; cervical groove very short, only forming two deep oblong depressions, directed inwards and backwards; lateral grooves indicated by an irregular row of punctations ending on a line posterior to the eyes, beginning again more outwardly posterior to the eyes and merging rapidly into a well-marked groove, continued so as to include the last two festoons. A few shallow punctations over the back; more distinct punctations on the raised areas between the posterior grooves. Very fine punctations evenly distributed, only few on festoons and on lateral borders. Festoons sharply defined.

Rostrum: *Basis capituli* broader than long, narrow behind, with the posterior and postero-lateral contours concave; antero-lateral margin straight. *Palps*: short, constricted basally; Articles 2 and 3 about equal in length, with article 3 having a slight external angle.

Ventral surface: Anus about midway along the length of the broadly sickle-shaped punctate *anal plates*, where incurved points face each other about $\frac{2}{3}$ along their length; accessory plates only slightly chitinated at their rounded tips. A rounded caudal process present.

Female. (Fig. 36.)

Unengorged 2.5 mm. \times 1.5 mm. to 3.75 mm. \times 2.5 mm.

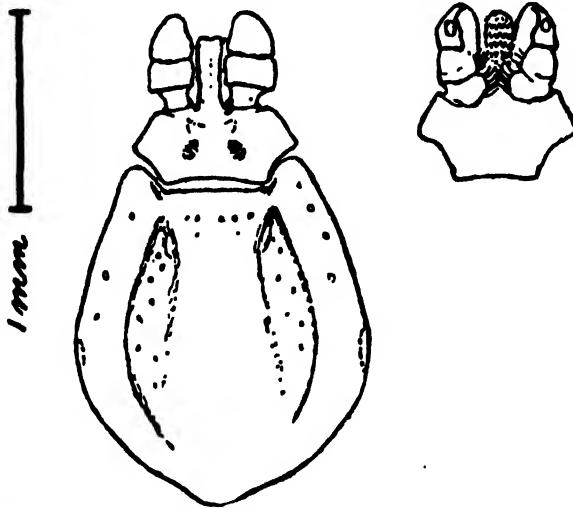


Fig. 36.—*R. simpsoni*, ♀, dorsal view and rostrum, ventral view, after Nuttall, 1910.

Scutum: longer than broad, 1.4 mm. \times 1.2 mm. to 1.8 mm. \times 1.6 mm., deeply emarginate, antero-lateral border but slightly convex; postero-lateral border sinuous, rounded posterior border with a slight median protrusion. Eyes pale, flat. Cervical and lateral grooves starting together in a deep pointed pit, the cervicals distinct for half the scutum length, then fading into a somewhat fusiform concavity lying between the median field and the raised lateral border, which latter is bounded internally by the lateral groove, this latter disappears near the posterior border. Punctations: a few scattered punctations, some coarser ones accentuating the lateral groove; as in the male fine punctations may be evenly scattered over the scutum.

Rostrum: *Basis capituli* resembles that of the male, but is somewhat broader, cornua faintly marked. Porose areas small, ovoid, directed obliquely forward and inward, distance apart equals twice the diameter.

Type: 5♂, 11♀ collected by J. J. Simpson off a large Rodent at Oshogbo; S. Nigeria.

Comment: Nuttall states "We at first referred the specimens to *R. falcatus* Neumann 1908, but on examining the types in the British Museum, and after consulting Prof. Neumann, we have decided to accord them specific rank. In *R. falcatus* the colour is blackish, the punctations numerous, the body and anal shields narrower. In the ♀ the scutum is as long as broad, 2 mm.; the punctations numerous. Owing, however to the great range of variability which my colleague, Mr. Warburton, and myself have observed in different species of *Rhipicephalus*, it is quite possible that some of the differences which we now regard as specific may ultimately prove to be merely varietal."

Geographical distribution: The Onderstepoort collection contains one batch of ticks off the edible rat *Thonomys swinderianus*, from N'buya, Uganda; and one lot off the same host from Nylstroom in the Transvaal. Bequaert 1931 reports it off *Aulacodus swinderianus* from the Congo da Lemba. The distribution of this species would thus seem to be that of its rodent hosts.

LITERATURE.

NUTTALL (1910). New species of ticks. *Parasitology III*, p. 413, fig. 6.

"*RHIPICEPHALUS SUPERTRITUS*" Neumann 1907.

Synonym R. coriaceus, Nuttall and Warburton 1907.

Male. (Figs. 37, 39.)

3½ mm. × 1½ mm. to 5½ mm. × 3½ mm. A large black species, which upon first sight gives the impression of a very coarse *R. appendiculatus*. Coxa I prominent dorsally.

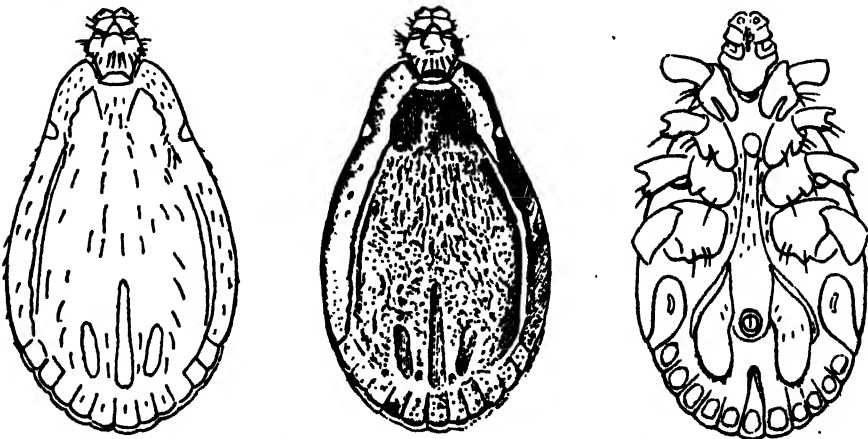


Fig. 37.—*R. supertritus*, dorsal and ventral view, after Nuttall and Warburton, 1907.

Conscutum: elongate, narrow in front, impressed at the level of the eyes. Eyes yellow, small, flat. Emargination fairly deep and narrow, its depth accentuated by the anterior prominence of coxa I. Cervical grooves convergent behind, marking off a narrow central field, their outer limits undefined, merging into a broad, flat, well-depressed area bounded by a ridge which, with an interruption, continues the lateral grooves anteriorly; surface of the depression covered with fine reticulations. Lateral groove clear-cut, deep, not picked out with punctations, usually ending clearly at first festoon, or going on less distinctly to include the second festoon or even part of the third. Posterior depressions: median groove elongate, the postero-laterals slightly broader and shorter; the surface of all three shows the same fine reticulate pattern as is seen in the lateral groove and in the cervical depression. Neumann (1907) mentions seven posterior grooves, but only figures five of about equal size, the two extra being to the outside of the postero-laterals. This tendency to the formation of extra reticulate depressions is also seen in the Lilongwe material, where slight depressions may form beyond the postero-laterals. These secondary depressions, however, are never well-defined, but they do show the fine reticulations. Festoons well-marked, decidedly longer than broad. Punctations: the greater part of the

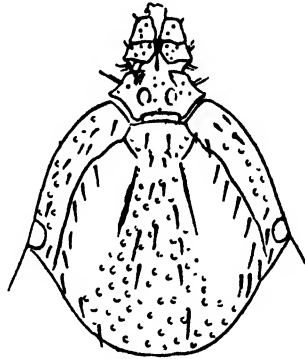


Fig. 38.—*R. supertritus* ♀, dorsal view after Nuttall and Warburton, 1907.

conscutum is covered with rugosities rather than with punctations, these rugosities may show varying degrees of roughness. Ordinary medium-sized punctations are present on the shoulders, on the lateral fields and on the festoons. Fine punctations are present on the ridges between the posterior grooves, and on the lateral strip, which lateral strip is usually quite smooth in *R. appendiculatus*. In some males the outline of the female scutum may be lightly picked out. Hairs on the shoulder, in the groove limiting the cervical field, in the lateral fields and along the lateral groove, and arranged in longitudinal rows in the posterior parts of the scutum, some specimens show a few fine hairs on the festoons. In the majority of specimens, however, most hairs are missing.

Abdomen: In fully engorged specimens the body may extend well beyond the conscutum, thus making visible the dark, ventral plaques, the penultimate being the largest. Beyond these, protruding from the ventral surface, are the threefinger-like protrusions of the body as figured by Neumann 1907. These arise further forward ventrally than does the single caudal appendage so characteristic of *R. appendiculatus*.

Rostrum: *Basis capituli*: Rather solid, $1\frac{1}{2}$ times as broad as long, cornua strong, postero-lateral margin concave; lateral angle in the anterior half; antero-laterals converging but slightly; a transverse row of long hairs continued on to the entero-lateral margins; surface uneven. *Palps* short and compact, with contour disturbed by the edges of articles 2 and 3; articles 2 and 3 flattened and hollowed dorsally, external margin edged with a raised border giving prominent postero-external angles. Article 1 slightly visible dorsally. Article 2 larger than article 3. The sub-collare (of Schulze 1935) well chitinized (not indicated as a separate element in Nuttall and Warburton's drawing).

Legs: stout. Leg 4 markedly larger than the other three, reddish brown rather than black.

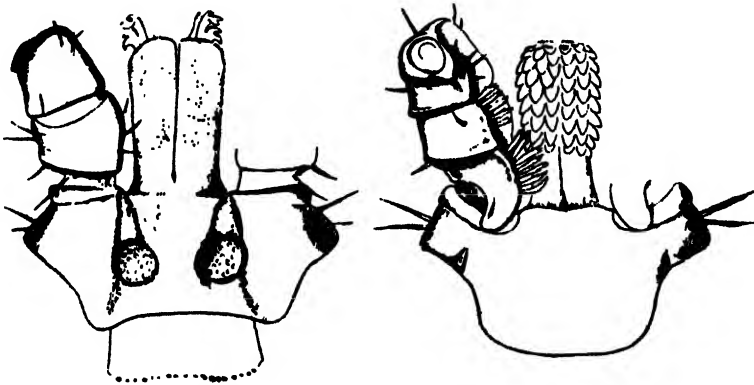


Fig. 39.—*R. supertritus*. Rostrum dorsal and ventral views, after Nuttall and Warburton, 1907.

Ventral surface: variable in colour, the three finger-like structures arising at the base of the anal plates usually somewhat orange. The eleven plaques highly chitinized and dark brown, the penultimate the biggest. **Anal plates:** not unlike those of *R. appendiculatus*, internal margin but slightly concave, external usually markedly convex, posterior margin rounded to bluntly pointed; the longest axis usually not down the centre of the plate as figured by Nuttall and Warburton, but as in *R. appendiculatus* approaching the internal margin. Accessory anals, elongate narrow, chitinized points.

Female. (Fig. 38.)

3.5 mm. \times 2.2 mm. to 5 mm. \times 3.3 mm., partially engorged.

Scutum: about as broad as long, dark brown; posterior margin slightly sinuous. Eyes flat, yellow, about halfway back. Emargination deep and wide. Cervical grooves short, pronounced, converging; fainter posteriorly and diverging; central field relatively narrow. Lateral groove pronounced reaching posterior margin, cervical depression pronounced, triangular, with reticulate surface, these reticulations forming a wider or narrower band following the lateral groove backwards. This reticulated area not nearly as extensive as indicated in Nuttall and Warburton's figure. Lateral groove seldom disturbed by punctations. Punctations tending to be coarse, coarsest

on the shoulders, present in the lateral field. In the central field they tend to be confluent, giving this central posterior area a rugose appearance. As in the male, short white hairs present.

Rostrum: *Basis capituli*, wider than long, cornua slight; postero-lateral margin slightly curved, longer than antero-lateral; antero-lateral short, constricted about midway; lateral angle in anterior half. Porose areas small, circular, more than their own diameter apart, touching externally a pronounced ridge; surface of central area uneven. A row of stiff, white hairs on lateral border reaching into antero-lateral margin, as in the male. *Palps*: surface uneven; contour slightly disturbed at junction of articles 2 and 3; dorsal surface slightly flattened, but not hollowed out as it is in the male; Article 2 slightly larger than 3. Article 2 broader than long. Article 3 squarish. Article 1 visible dorsally.

Legs: Not as stout as in the male, equal, reddish brown.

Dorsal and Ventral Surface: with longitudinal rows of sparse hairs; festoons well marked both dorsally and ventrally.

Comments: *R. supertritus*, female, could easily be confused with *R. sinus* or with *R. tricuspis*. It can be readily distinguished from these two species by the reticulated cervical field and the reticulated lateral groove, and by the fact that in *R. sinus* and *R. tricuspis* the shorter lateral grooves are always picked out with punctations. In *R. supertritus* the surface of the *basis capitulum* is uneven, a row of stiff hairs runs parallel with the postero-lateral margin, whereas the surface is smooth and the row of hairs is absent in the other two species.

Type: *R. supertritus*. 2♂ from a horse on the banks of the Lualaba, Belgian Congo.

R. coriaceus 2♂, 6♀, collected by Dr. Old in North Nyasaland in 1907, and 1♂ collected by Dr. Wellman in the Benguella hinterland in 1907.

Neumann (1908) supplemented his original descriptions after 10♂, 9♀ collected at the North end of Lake Nyasa by Dr. Old, deposited in the British Museum.

The revised description of *R. supertritus* given above is based on three batches most kindly placed at my disposal by S. G. Wilson of the Veterinary Department at Lilongwe, Nyasaland.

Geographical Distribution.—Besides the above four *type* records, Bequaert 1931 records it off *Taurotragus derbianus gigas*, the Eland, from Garamba, Belgian Congo. The Lilongwe batches consist of 12♂ and 15♀ off Buffalo at Chinunka, Songwe River, N. Nyasaland, collected 21.12.44; and 3♂ off a Sable Antelope at Rusa River, Fort Manning, on 30.8.44, all in Northern Nyasaland.

LITERATURE REFERENCES.

- NEUMANN (1907). Notes sur les Iodides V. *Archives de Parasitologie* XI, p. 215.
 NEUMANN (1908). Notes sur les Ixodidae VII. *Notes from the Leyden Museum*, Vol. XXX, p. 79.
 NUTTALL AND WARBURTON (1907). On a new genus of the Ixodidae together with a description of eleven new species of ticks. *Proceedings of the Cambridge Philosophical Society*, Vol. XIV, 1908.

RHIPICEPHALUS THEILERI Bedford and Hewitt 1925.

Male. (Fig 40.)

Body oval, narrow in front, widest at level of coxa IV. 3 mm. \times 2 mm.

Conscutum: Reddish brown. Eyes small, flat. Cervical groove short and deep; lateral groove includes one festoon and extends forwards as far as the cervical; posterior median and lateral grooves absent; festoons short, coarsely punctate and thus somewhat obscured. Punctations numerous and large except anteriorly where they are less numerous and mostly fine. On each side of the median line in the middle there is a shallow groove formed of punctations, and besides these in the posterior half of the scutum another but shorter pair of shallow longitudinal depressions more mesially situated, and thereon likewise are numerous punctations, whilst between them the median region presents an incipient ridge free of punctations.

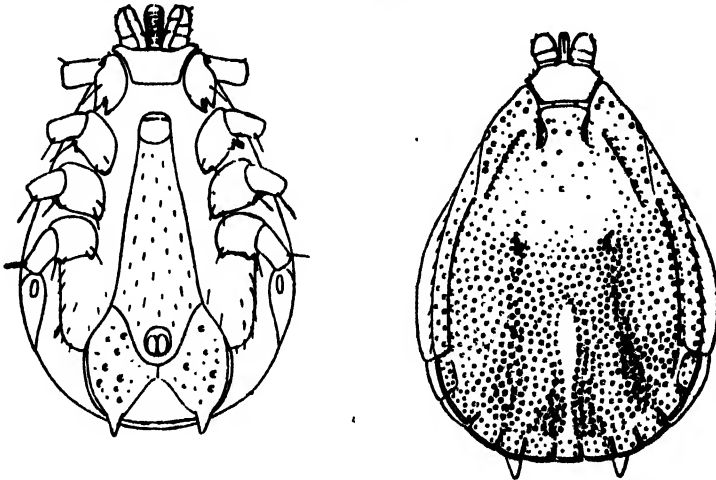


Fig. 40.—*R. theileri* ♂. dorsal and ventral views. Bedford, del.

Rostrum.—*Basis capituli* more than three times as broad as long, lateral angles acute, cornua slight, with a few fine punctations. *Palps*: Article I visible dorsally, article 2 and 3 about the same size.

Legs.—Pale brown, anterior projection of coxa I not prominent when viewed dorsally.

Ventral Surface.—Reddish brown throughout, the whole surface being chitinized more or less strongly. *Anal plates*: extending well in front of anus, large, pointed posteriorly, their apices visible from above, external margin convex; posterior drawn out into a point; internal with a projection inwards behind the anus; surface coarsely punctate. Well developed accessory plates are not present, but the place where such shields often occur is swollen over a considerable area and rather deeply coloured, yet lateral and posterior borders are not defined.

Female. (Fig. 41.)

Body oval, when unengorged gives hairy appearance of *I. pilosus*.

Scutum.—Dark brown, posterior margin rounded, 1 mm. \times 1.5 mm. Cervical groove: the anterior deep portion is elongate, with straight internal edge and convex external edge converging; the cervical groove generally does not show a shallow superficial extension backwards, but may do so; so that in most instances there is no cervical field. Lateral grooves formed of a row of large pits. Punctations scattered, unequal, in central field somewhat superficial, or may be more pronounced giving a fleeting impression of *R. sanguineus*; generally a row of large punctations on lateral border. Scutum at first sight may be mistaken for *R. simus*.

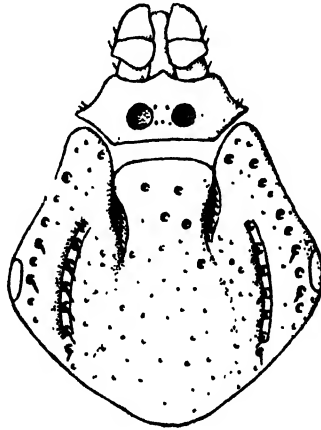


Fig. 41.—*R. theileri*, dorsal view, after Bedford and Hewitt, 1925.

Rostrum.—Most characteristic feature. *Basis Capituli* about three times as broad as long. Posterior edge straight, with short rounded blunt cornua; postero-lateral margins concave; longer than the antero-laterals; widest in anterior third; antero-lateral margins sinuous, concave at first then convex before it meets the base of the palps, meeting with the postero-lateral margin in a fairly sharp point, and forming a ledge, which latter is sometimes even more pronounced than in Bedford's figure. Sometimes antero-lateral margin is almost a straight line (giving the impression of *R. sanguineus*). Ventrally this ledge swings in to form an auricula. *Palps* broad. Article II widest about $\frac{2}{3}$ of the way up, from here to the top of article 3 the internal contour is concave; in some instances, however, it is almost straight.

Ventral surface: sparsely clothed with short white hairs, longest and most numerous along the posterior margin.

Legs: reddish brown.

Type: 1♂, 1♀ from ground squirrel, *Geosciurus capensis*, collected by R. Bigalke at Glen, Orange Free State in 1921. Deposited at Onderstepoort. At present on loan to Dr. Schulze at Rostock.

Occurrence: The Onderstepoort collection has batches of females mostly off small burrowing mammals from the Orange Free State, viz., five batches off *Suricata suricata hameltoni*, meercat, from the farm Vaalbank in the Edenburg district; one batch off a suricate on the farm Rooidam in the Jacobsdal district; two batches off *Cynictis penicillata ogilbeyi*, yellow mongoose, from Vaalbank, Edenburg; one batch off *Geosciurus capensis* from Rooidam, Jacobsdal; 2 batches off the yellow mongoose, also from Rooidam; one male off *Vulpes chama*, silver jackal, from Petrusburg.

The above collections were all made in connection with a drive to eradicate rabies from certain areas in the Free State, and represent the tick catch off a vast number of small carnivores and ground squirrels. From the information thus made available it would seem that the tick is never abundant and that in all probability it is not confined to the Orange Free State, as the above catches seem to indicate, but that its geographical distribution is that of its commoner hosts, the Viverridae, which are frequently found associated with the ground squirrels.

LITERATURE REFERENCE.

EDFORD AND HEWITT (1925). South African ticks. *The S.A. Jnl. Nat. Hist.*, Vol. V, p. 263, fig.

“ RHIPICEPHALUS TRICUSPIS ” Dönitz 1906.

Synonym: R. lunulatus Neumann 1907.

R. glyphis Dönitz 1910.

R. sinus var. *lunulatus* Warburton 1912.

Male. (Fig. 42.)

Up to 4 mm. in length; reddish to dark brown. Narrow in front, widening rapidly behind the eyes. Coxa I barely or not at all visible dorsally.

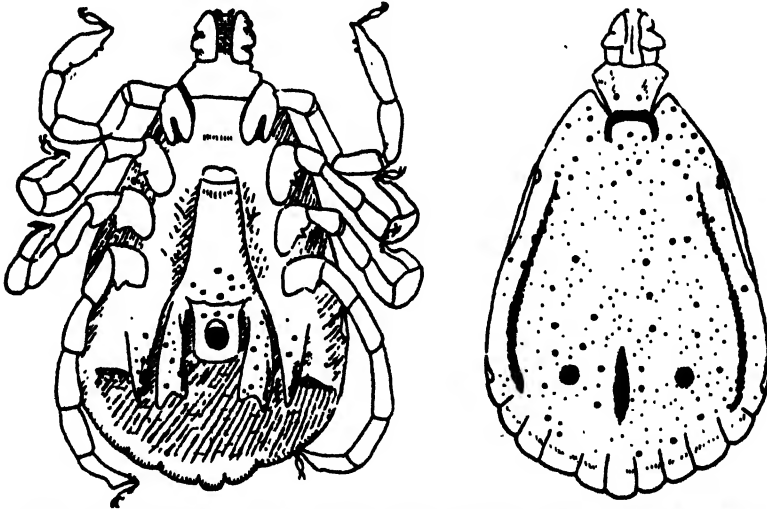


Fig. 12.—*R. tricuspis* ♂, dorsal and ventral views Dönitz, 1906.

Consutum: Eyes flat, light coloured, small, sometimes difficult to distinguish. Cervical grooves: short deep. Lateral grooves well developed, commencing a short distance behind the eyes; may include the first festoon, picked out with large punctations; preceded anteriorly by a row of 3 to 4 large punctations situated slightly further inwards and extending to level of cervical grooves. Posterior median groove varying in shape from a straight line to a narrow spindle; the laterals are in the form of small circular depressions. In the unfed specimens the posterior portion of the scutum usually

shows three long depressions reaching up to midway; these undoubtedly represent the median and paramedian muscular attachment grooves; depressions are sometimes also seen in the anterior paramedian position. Punctations, small punctations, finer posteriorly than anteriorly distributed fairly evenly, extending on to the lateral border and the festoons, though not so numerous in these last two areas. Large punctations present, six to eight posterior to the grooves, about two external to the postero-laterals; a row of four to five on either side of the median; scattered fairly evenly but very far apart between the posterior grooves and eye level, more closely clustered on the central anterior field and on the shoulders. The punctations give the conscutum an uneven and untidy appearance.

Rostrum: Basis capituli, more than twice as broad as long; postero-lateral margin about twice as long as the antero-lateral. Middle portion of the basis capituli sunken, flanked on either side by a dorsal ridge ending posteriorly in a short broad cornua. *Palps* as broad as long. Article 1 visible dorsally. Article 2 broader than long, internal margin longer than external. Article 3 longer than 2, as long as broad, hollowed out somewhat dorsally.

Ventral surface: Anal plates: external and internal margins almost straight, running practically parallel distally, and usually about equally long; posterior margin concave, forming a point where it meets the external and where it meets the internal margins. The inner points usually heavier and blunter, the external lighter and sharply pointed. These points, however, vary considerably in relative length, they may be equal or the outer or the inner may be decidedly long (in the descendants of one female). Only the tip of the accessory is usually chitinized, mostly as a fairly sharp point.

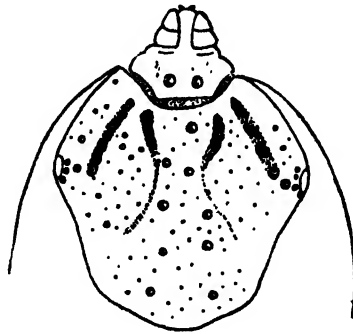


Fig. 43.—*R. tricuspis* ♀, dorsal view after Dönitz, 1906.

Female (Fig. 43.)

Scutum: reddish brown to dark brown; as broad as long, or slightly longer than broad, widest at eye level about midway; postero-lateral margin sinuous, posterior margin frequently slightly pointed. Cervical grooves deep, narrow, converging, followed by shallow divergent depressions extending about two-thirds of the way backwards. Lateral grooves well developed, picked out with large punctations extending almost to the edge of the scutum. Punctations: numerous small punctations, the same size as the anterior punctations of the male, evenly dispersed over the entire scutum, the lateral fields included. A few large punctations scattered over the scutum, somewhat closer together anteriorly and on the shoulders. The cervical field is sometimes finely rugose. The punctations give the scutum an uneven untidy appearance, well illustrated in Dönitz, figure.

Rostrum: short. **Basis capituli:** twice as long as broad, lateral angles prominent, midway; postero-lateral margin concave, antero-lateral straight until it meets the base of palps, then convex; cornua short but stout; central field sunken as in the male, porose areas broad, oval; **Palps** broader than long. Article 2 broader than long; Article 3 longer than 2, about as long as broad.

Nymph. (Figs. 44–45.)

Scutum: as broad as long, widest at eye level in the posterior three-fifths; antero-lateral margin straight, postero-lateral evenly curved. Emargination shallow. Cervical groove and lateral groove long with a depressed cervical field between them.

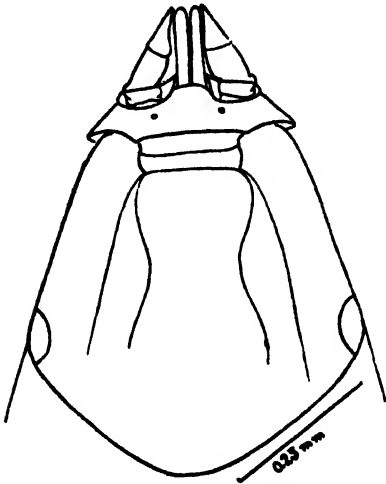


Fig. 44.

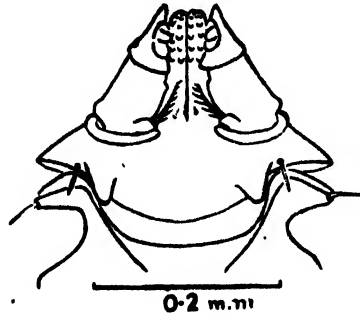


Fig. 45.

Fig. 44.—*R. tricuspis* nymph, dorsal view. D. Pringle, del.

Fig. 45.—*R. tricuspis* nymph, ventral view. D. Pringle, del.

Rostrum: triangular with base of the triangle greater than its height, in the proportion of 3:2. **Basis capituli:** short, six times as wide as long; lateral angles far back, acute, and giving the appearance of pointing backwards, antero-lateral margin a straight line, postero-lateral but slightly concave; no cornua; basis capituli overlaps anterior margin of the body. On the ventral surface a well developed spur present at the junction between the auricula and the posterior margin. Palps with outer contour a straight line, the two sides converging to form an acute apex; Article 2 and 3 longer than broad, article 2 much longer than article 3, article 3 triangular, with outer margin longer than inner margin. Article 1 not visible dorsally.

Larva. (Figs. 46–47.)

Scutum decidedly broader than long, broadest at eye level, very far back. Emargination shallow. Antero-lateral margin long and straight; postero-lateral and posterior-margins but slightly curved giving the scutum a truncated appearance. Cervical depression reaching fairly far back.

Rostrum: Triangular, broader than long, approximately as 3:2. **Basis capituli** about four times as broad as long; lateral angles vaguely rounded, prominent; postero-lateral margin slightly concave, longer than antero-lateral; antero-lateral slightly sinuous. Ventrally a spur present at the junction of the auricula and the posterior margin. **Palps:** external contour a straight line, palps leaning towards one another; dorsally it is difficult to differentiate between articles 2 and 3; widest at the base. Ventrally aricle 3 has a retrograde spur.

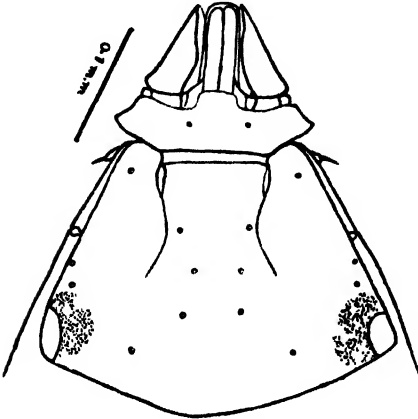


Fig. 46.

Fig. 46.—*R. tricuspis* larva, dorsal view. D. Pringle, del.

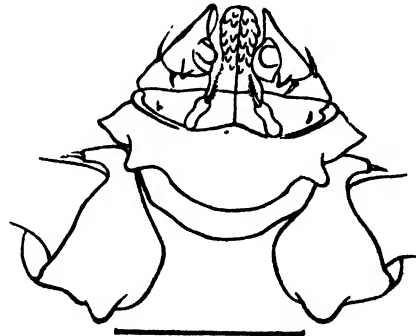


Fig. 47.

Fig. 47.—*R. tricuspis* larva, ventral view. D. Pringle, del.

Type: *Tricuspis*: free at Lehututu Kong in the Kalahari.

Lunulatus: 2♂ off a horse, on the banks of the Lulaba river, Belgian Congo; deposited in the British Museum.

The drawings of the nymph and of the larva are from material reared at Onderstepoort from eggs of a female collected at Pretoria North.

Occurrence: The geographical distribution of *R. tricuspis* is difficult to establish at this stage, since most of the recent workers have considered it as a synonym of *R. simus*. It has, however, been recorded as *R. tricuspis* by Nuttall 1916 and by Schwetz 1927 from the Belgian Congo; as *R. glyphis* by Dönitz 1910 off a *Potamochoerus* from Tanganyika and off cattle from Togoland; the Onderstepoort collection contains batches off *Lepus capensis* from the Hoopstad district in the Orange Free State; off bovines from Chikamula, Southern Province Nyasaland, collected by S. G. Wilson. (Mr. Wilson has since found it on a wide variety of hosts all over the Northern Province); off bovines from the Mongu area, Borotseland, collected by P. L. le Roux; and off sheep at Pretoria North. Neumann in "Das Tierreich" 1911 lists it as also having been collected off *Erinaceus frontalis*.

The Zoological Survey has records of *R. tricuspis* off domestic stock from farms near Ofcolaco, Gravelotte, and Duivelskloof in the Letaba district, Northern Transvaal; from farms on the Sabie- and the White-River Plateaux; in the De Kaap Valley in the Schoemanskloof area, from Kaapschehoop and Schoonoord on the edge of the Highveld, and from the Lowveld near Nelspruit in the Barberton and Nelspruit districts of the

Eastern Transvaal; from the farms Welbedacht, near Paulpietersburg; Waterfalls, near Richmond; Sweetwaters, near Ixopo and from farms in the Entonjaneni district of Natal; from farms in the Bizana district of Pondoland; from Gannaoor in the Vryburg district; from the farms Thanoanche, Nyra end Esperanza in the Kuruman district of Bechuanaland, i.e., it occurs in the warmer parts of South Africa, in areas containing thorn trees, varying from the semi-arid bushveld of the Kalahari to the moister bushveld of the lowveld of the Northern- and of the Eastern-Transvaal and of Natal. It is absent from the scrubveld of the Karroo and from the open grassveld typical of the Orange Free State and also of the middle and of the high-veld of the Transvaal.

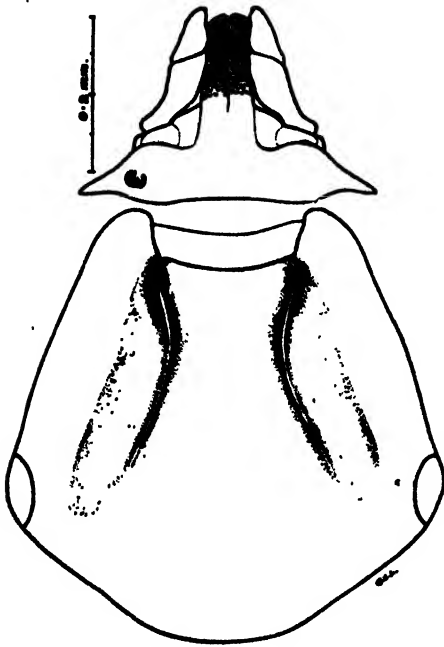


Fig. 48.

Fig. 48.—*R. simus* nymph, dorsal view, after Theiler, 1943.

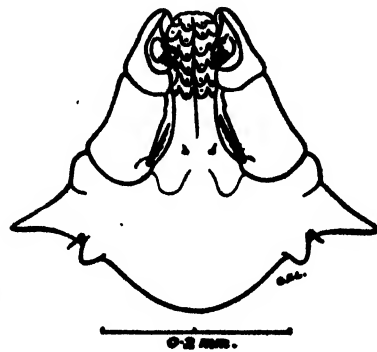


Fig. 49.

Fig. 49.—*R. simus* nymph, ventral view, after Theiler, 1943.

Classification.

Comments: This species was first described as *R. tricuspis* by Dönitz 1906 and as *R. lunulatus* by Neumann 1907. Dönitz 1910 describes his *R. glyphis*. Neumann 1911 in his monograph on the Ixodidae sinks *R. lunulatus* as a synonym of *R. tricuspis* but does not list *R. glyphis* among the known Rhipicephalids. Warburton 1912 overlooks Dönitz' publications as well as Neumann's 1911 monograph and sinks *R. lunulatus* as a variety of *R. simus*, and as such it has remained to most workers

The study of the descendants of one female collected off a sheep at Pretoria North and reared till the F₂ generation at Onderstepoort, however, clearly shows *R. tricuspis* to be a valid species. The descendants all conform to type and show the usual minor differences as seen in the various Rhipi-

cephalid species which have been studied in detail thus far, differences which cover all the variations described for *R. tricuspis*, *R. lunulatus* and *R. glyphis*, but which never approach *R. simus*.

R. simus differs from *R. tricuspis* in that:—

- 1 *R. simus* ♂ is a larger, heavier tick; *conscutum* shiny, dark brown to black; the cervical and lateral grooves clean and clear cut; the small punctations are fine and superficial or even absent, the large punctations are deep and clear-cut often containing short white hairs; the *basis capituli* is more compact, with the lateral angle blunter. *Anal plates*, external and posterior margin curved, i.e., anal plate bean to kidney-shaped. *Legs* stout.

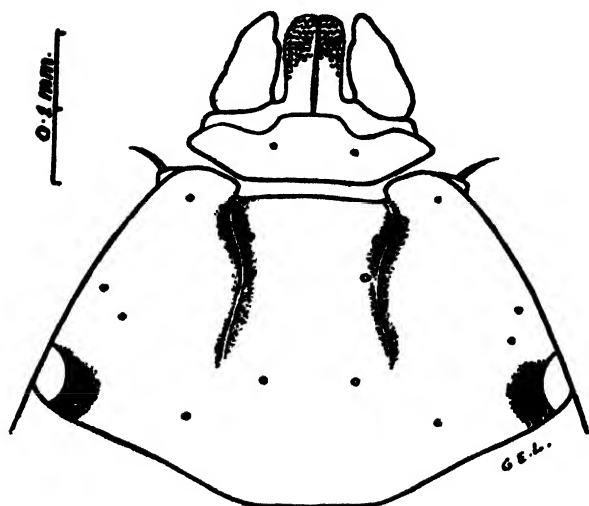


Fig. 50.

Fig. 50.—*R. simus* larva, dorsal view, after Theiler, 1943.

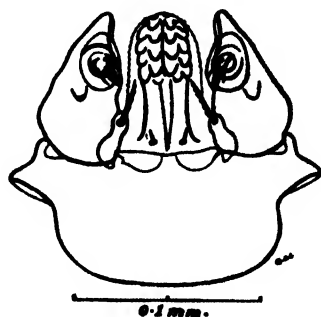


Fig. 51.

Fig. 51.—*R. simus* larva, ventral view, after Theiler, 1943.

- 2 *R. simus* ♀ is a larger, heavier tick; *scutum* subcircular; shiny, prominent, raised lateral border with but few light punctations; punctations present the same neat picture as in the male, the fine punctations, however, are a little heavier and the large punctations not so prominent. *Basis capituli* heavier with the lateral angles blunter.
3. *R. simus*: *Nymph*: (Figs. 48–49) *basis capituli* relatively broader with the lateral angle sharper and extending well beyond the scutum.
4. *R. simus*: *Larva*: (Figs. 50–51) *basis capituli* relatively broader; no spur attached to ventral surface.
5. S. G. Wilson at Lilongwe, Nyasaland [private correspondence] finds that there is a marked difference in the habits of these two ticks. *R. tricuspis* appears and is found in considerable numbers during late November to early February in collections off cattle, being confined to the tail switch; whereas *R. simus* is not a cattle tick and its main active season appears to be January to May.

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- DÖNITZ (1910). Zwei neue afrikanische Rhipicephalus Arten (*R. glyphis*). Sitzb. Ges. Natf. Freunde, Berlin, VI, p. 278, fig.
- NEUMANN (1907). Notes sur les Ixodides V. Arch. Parasit, XI, p. 215, fig.
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- WARBURTON (1912). Notes on the Genus Rhipicephalus. Parasitology, V. p. 3.

RHIPICEPHALUS ZIEMANNI Neumann 1903.

Synonym: *Rhipicephalus cuneatus* Neumann 1908.

Male. (Figs. 52–53.)

Body elongate narrow; twice as broad posteriorly as anteriorly, curved posteriorly: 4·25 mm. long (with rostrum): 2·3 mm. broad behind the posterior third.

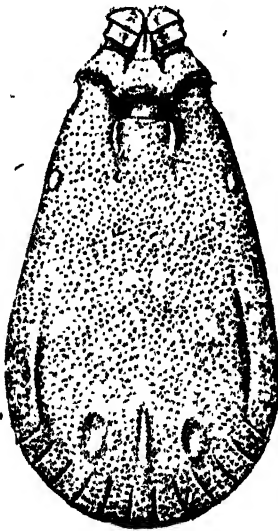


Fig. 52.

Fig. 52.—*R. ziemanni* ♂, dorsal view, after Neumann, 1908.

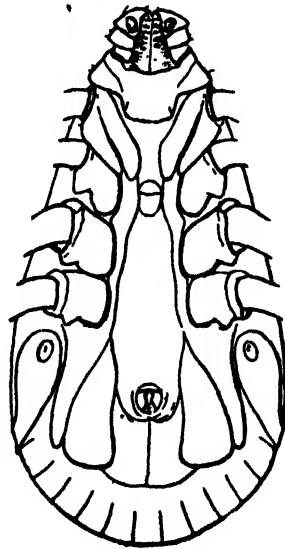


Fig. 53.

Fig. 53.—*R. ziemanni* ♂, ventral view, after Neumann, 1908.

Consutum: slightly convex; as shown in the figure the scapulae are prolonged into a curved protuberance pointing outwardly; shiny; chestnut brown, inornate, festoons slightly lighter in colour. Eyes flat, medium-sized, yellowish; marginal, situated slightly in front on the anterior third. Emargination as in the figure. Cervical groove very short, deep. Lateral grooves

shallow, sometimes almost obsolete, starting slightly in front of the anterior half and ending in the last festoon. Posterior grooves present, short and wide, the median longer. Punctations very numerous, medium, subequal.

Rostrum: 0.65 mm. *Basis capituli* about twice as broad as long, with punctations; lateral angles prominent towards the middle of the margin; cornua prominent (not very distinct in the figure). *Palps*, rather shorter than the basis, hardly longer than broad, flat on the dorsal surface. Article II well separated from the basis (i.e., article I visible dorsally); as long as article III, rectangular.

Legs: strong and long. Coxa I not visible dorsally.

Ventral surface: reddish brown, lighter posteriorly. Anus towards the anterior third of the anal plate. *Anal plates* triangular, numerous punctations, posterior margin slightly convex; external margin slightly convex; internal margin slightly concave. *Accessory* anals chitinous, well developed. No caudal process.

Female. (No. Fig.)

5 mm. \times 3 mm. to 8 mm. \times 5 mm. engorged. *Scutum* as broad as long, 1.8 mm., with margins but slightly sinuous. Cervical grooves short, deep, narrow, concave internally. Lateral grooves absent. Punctations numerous, fine, subequal, evenly dispersed. Eyes flat, large, yellowish.

Rostrum: *Basis capituli* more than twice as broad as long. Porose areas oval, longer than broad, distance apart equal to their width.

Legs: stout, punctate.

Type: *Ziemanni* 13♂ and 19♀ collected off a cow by Ziemann in the Cameroons.

Cuneatus 3♂ collected off cattle by Pelat at Ngomo on the Ogooué in the French Congo. Deposited with Prof. Galli Valerio at Lausanne.

Comments. Bequaert 1930 records 1 *R. cuneatus* off a monkey in Liberia with this remark: "This specimen agrees in every detail with the original figures and description. I have my doubts, however, as to the specific distinctness of *R. cuncatus* and *R. ziemanni* (off cattle, Cameroons). The latter has also been recorded by Neumann from Liberia, but since it has not been figured, I hesitate to unite the two species."

The only other Rhipicephalid which shows the same peculiar elongation of the scapular region, as is seen in this species, is *Rhipicephalus* (*Pterygodes*) *fulvus* Neumann 1913 from Tunis. In *Pterygodes fulvus*, however, the shoulder is very much longer with a more pronounced outwardly curved tip. This elongation of the scapular region is, however, not developed in the female, in the nymph nor in the larva as described by Colas-Belcour 1932. Hence one can assume that this peculiarity will also be absent in the female of *R. cuneatus* or *R. ziemanni*.

Bequaert is somewhat hesitant to sink *R. cuncatus* as a synonym of *R. ziemanni*. The descriptions of the males are, however, so very similar as to make it impossible to find any difference that is of specific importance. So that one can but assume that *R. cuneatus* is the same as *R. ziemanni*.

LITERATURE REFERENCES.

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 NEUMANN (1908). Notes sur les Ixodides, Note XI (*R. cuneatus*). *Notes from the Leyden Museum*, XXX, p. 76, figs.
 NEUMANN (1913). Un nouveau sous-genre et deux nouvelles espèces d'Ixodides (*P. fulvus*). *Bull. Soc. Zool. France, Paris*, XXXVIII, p. 147, figs.

SUMMARY.

1. Omitting unessentials, the descriptions of various little known Rhipicephalids and their geographical distribution have been republished.

2. The descriptions of *R. distinctus*, *R. masseyi*, *R. theileri*, *R. tricuspis*, *R. simpsoni*, *R. supertritus* are brought up to date.

The nymphs of *R. masseyi*, *R. tricuspis*, *R. distinctus* and the larva of *R. tricuspis* are described for the first time.

3. *R. falcatus* and *R. tricuspis* are shown to be valid species.

4. *R. attenuatus* is sunk as synonymous with *R. masseyi*; *R. cuneatus* as *R. ziemanni*; *R. lunulatus* and *R. glyphis* as *R. tricuspis*; *R. schwetzi* as *R. dux*.

5. Dönitz' supposition that the occurrence for *R. foliis* is "domestic stock, South Africa", is confirmed with reservations.

6. A list of the commoner African Rhipicephalids, whose descriptions are not included in this article, is given.

LITERATURE RÉFÉRENCES.

The relevant publications are given at the end of the description of each species.

The Supplementary Effect between the Proteins of a Heat-Treated Soybean Meal ("Soma Meal") and a South African Strain of Yellow Maize Seed ("Eksteen").

By S. J. MYBURGH, Section of Biochemistry and Nutrition,
Onderstepoort.

INTRODUCTION.

It has been shown by Marais (1940) amongst others, that yellow maize protein and soya bean meal protein supplement each other in tests carried out with rats. In the present study, a yellow maize seed variety called Eksteen was selected. This seed is a small, flint type, deep orange-red in colour and is one of several excellent varieties improved by selective breeding at the Summer Rainfall Cereal Experimental Station at Kroonstad in the Orange Free State (season 1941-1942). The soybean meal is a product kindly supplied by the Delmas Milling Company, Transvaal. Both these products have been separately studied and the biological values found to be on an average 63.3 for soybean meal and 69.9 for the maize seed at 8 per cent. protein level in the ration of rats (Myburgh, 1946).

The objects of this study were to determine whether these proteins had any supplementary effect on each other, and furthermore, if the two different salt mixtures used in the rations had any effect on the digestive processes and the biological values. The salt mixtures were those recommended by Hubbel *et al* (1937) and Hawk and Oser (1931). Both these mixtures of salts have been used in metabolism studies at this and other Institutes.

EXPERIMENTAL.

One biological value only was determined for each of the two rations indicated in Table 1, using two sets of Wistar rats. The detailed technique of Marais and Smuts (1940) of the method described by Mitchell (1924) was followed, except for the following modification: the N-low period followed the protein period. This procedure was also followed in previous tests. It has been found by Miller (1942) and by Myburgh (unpublished data) at this Institute, that when the N-low period precedes the protein period, higher biological values are obtained, hence the modification was an improvement. The results of this study are given in Tables 2 and 3.

RESULTS.

The biological values of the maize and soybean meal determined separately, were found to be 69.9 and 63.3 respectively, as previously stated. The average biological value for the combination of the proteins was 73.5. There was therefore a small supplementary effect. This biological value is approximately the same as that obtained by Marais and Smuts (1940), namely 75.

SUPPLEMENTARY EFFECT BETWEEN SOYBEAN MEAL AND YELLOW MAIZE SEED.

The choice of the salt mixture, either that of Hubbel or of Hawk and Oser, did not affect the digestion or metabolism of the protein, as indicated by the identical biological values and almost identical true digestibilities.

SUMMARY.

1. The proteins of an improved yellow maize strain popular in South Africa and that of a heat-treated soybean meal, supplemented each other to a small extent.

2. There was no determinable difference in the digestion or metabolism of the protein, when either the Hubbel or the Hawk and Oser Salt Mixtures were used in the rations with identical proteins.

LITERATURE.

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- MYBURGH, S. J. (1944). The nutritive value of the protein of a few South African soybean meals. *Onderstepoort J.*, Vol. 19, No. 1 and 2, p. 161.
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TABLE 1.
Composition of the Rations.

Ingredients.	N.—Low Ration.		Ration A.	Ration B.
	Hubbel.	Hawk & Oser.	(Hubbel Salts.)	(Hawk & Oser Salts.)
Somameal.....	—	—	10·0	10·0
Eksteen.....	—	—	40·0	40·0
Sucrose.....	10·0	10·0	10·0	10·0
Butterfat.....	8·0	8·0	8·0	8·0
Harris Vit.B.....	2·0	2·0	2·0	2·0
Cod Liver Oil.....	2·0	2·0	2·0	2·0
Hubbel Salts (1).....	2·0	—	2·0	—
Hawk & Oser Salts (2).....	—	4·5	—	4·5
NaCl.....	1·0	1·0	1·0	1·0
Dextrinized Starch.....	69·2	66·7	25·0	22·5
Whole Egg (Ether-extracted).....	3·8	3·8	—	—
Agar.....	2·0	2·0	—	—
TOTAL.....	100·0	100·0	100·0	100·0
% N.....	0·6	0·6	1·45	1·46

(1) *Science* (1931) 74,369. (2) *J. Nutrition* (1937) 14,273.

TABLE 2.

Nitrogen Metabolism Data. Calculation of the Biological Value.

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Nett N—Utilization.
							Per Gm. Food.	Per Day.				Per 100 Gm. Weight.	Per Day.							
1	105	114	110	7.0	101.5	23.9	2.31	16.2	7.7	83.8	44.6	18.4	20.2	24.4	68.4	33.0	76.5	92.4	74.0	68.4
2	106	115	111	7.5	108.7	25.6	2.01	15.1	11.5	97.2	46.0	16.8	18.6	27.4	68.8	37.1	76.5	89.6	71.9	64.5
3	107	114	111	7.5	108.7	21.4	2.01	15.1	6.3	102.4	41.8	15.7	17.4	24.4	78.0	45.5	80.2	94.1	76.2	71.6
4	115	122	119	8.0	116.0	20.9	2.40	19.2	1.7	114.3	50.1	19.0	22.6	27.5	86.8	45.0	81.8	86.6	76.1	75.1
5	120	127	124	8.0	116.0	24.1	2.30	18.4	5.7	110.3	52.4	14.5	18.0	34.4	75.9	39.5	79.3	95.2	68.9	65.6
6	99	107	103	7.0	101.5	23.52	2.30	16.1	7.4	94.1	42.8	17.6	18.1	24.7	69.4	35.2	77.0	92.18	73.8	68.4
AVERAGE.....																	78.5	93.8	73.5	68.9

Whole Maize (Eksteen)—Sonameal "Ration A" (N = 1.45 per cent.).

N-low Period.

1	116	120	118	7.7	—	17.8	2.31	—	—	—	—	21.7	18.4	—	—	—	—	—	—	—
2	116	121	119	7.7	—	15.45	2.01	—	—	—	—	20.0	16.8	—	—	—	—	—	—	—
3	118	121	120	7.7	—	15.45	2.01	—	—	—	—	18.8	15.7	—	—	—	—	—	—	—
4	128	128	128	8.2	—	19.7	2.40	—	—	—	—	24.3	19.0	—	—	—	—	—	—	—
5	133	138	136	8.7	—	20.0	2.30	—	—	—	—	19.65	14.5	—	—	—	—	—	—	—
6	111	117	114	7.2	—	16.55	2.30	—	—	—	—	20.0	17.6	—	—	—	—	—	—	—

TABLE 3.
Nitrogen Metabolism Data. Calculation of the Biological Value.

Rat No.	Initial Weight.	Final Weight.	Average Weight.	Daily Food Intake.	Daily N Intake.	Daily Faecal N.	Metabolic N.		Food N in Faeces.	Absorbed N.	Daily Urinary N.	Endogenous N.		Food N in Urine.	Retained N.	N Balance.	Apparent Digestibility.	True Digestibility.	Biological Value.	Percentage Net N—Utilization.
							Per Gm. Food.	Per Day.				Per 100 Gm. Weight.	Per Day.							
Whole Maize (Eksteen)—Somameal " Ration B " (N = 1.46 per cent.).																				
7	103	111	107	7.0	102.2	21.9	Mgm. 2.21	Mgm. 15.4	Mgm. 6.5	Mgm. 95.7	Mgm. 46.8	Mgm. 16.6	Mgm. 17.7	Mgm. 29.1	Mgm. 66.6	Mgm. 33.5	78.6	93.6	69.6	65.2
8	112	120	116	8.0	116.8	27.1	Mgm. 2.24	Mgm. 17.9	Mgm. 8.2	Mgm. 107.6	Mgm. 52.0	Mgm. 18.6	Mgm. 21.6	Mgm. 30.4	Mgm. 77.2	Mgm. 37.7	76.8	92.2	71.8	66.2
9	102	109	106	7.0	102.2	23.4	Mgm. 2.02	Mgm. 14.1	Mgm. 9.2	Mgm. 92.9	Mgm. 39.6	Mgm. 16.8	Mgm. 17.8	Mgm. 21.8	Mgm. 71.1	Mgm. 39.2	77.1	91.0	70.6	69.8
10	131	140	136	8.5	124.1	25.3	Mgm. 2.13	Mgm. 18.1	Mgm. 7.2	Mgm. 116.9	Mgm. 54.1	Mgm. 18.7	Mgm. 25.4	Mgm. 28.7	Mgm. 88.2	Mgm. 44.7	79.6	94.3	75.5	71.2
11	90	98	94	7.0	102.2	24.1	Mgm. 2.74	Mgm. 19.1	Mgm. 5.0	Mgm. 97.2	Mgm. 47.2	Mgm. 21.2	Mgm. 19.9	Mgm. 27.3	Mgm. 59.9	Mgm. 30.9	76.4	95.1	72.0	68.6
12	91	100	96	7.0	102.2	25.2	Mgm. 2.12	Mgm. 14.8	Mgm. 10.4	Mgm. 91.8	Mgm. 38.4	Mgm. 16.5	Mgm. 15.8	Mgm. 22.6	Mgm. 69.2	Mgm. 38.6	75.4	90.0	75.4	68.0
AVERAGE.....																	77.3	92.7	73.5	68.2
N-low Period.																				
7	116	121	119	7.2	—	15.9	Mgm. 2.21	—	—	—	—	19.8	Mgm. 16.6	—	—	—	—	—	—	—
8	123	126	125	8.2	—	18.4	Mgm. 2.24	—	—	—	—	23.2	Mgm. 18.6	—	—	—	—	—	—	—
9	117	121	119	7.7	—	15.6	Mgm. 2.02	—	—	—	—	20.0	Mgm. 16.8	—	—	—	—	—	—	—
10	144	152	148	8.7	—	18.5	Mgm. 2.13	—	—	—	—	27.7	Mgm. 18.7	—	—	—	—	—	—	—
11	96	100	98	6.5	—	17.8	Mgm. 2.74	—	—	—	—	20.8	Mgm. 21.2	—	—	—	—	—	—	—
12	103	109	106	6.7	—	14.2	Mgm. 2.12	—	—	—	—	17.5	Mgm. 16.5	—	—	—	—	—	—	—

Notes on the Thiocyanate Iron Reaction.

A Modified Procedure for the Quantitative Determination of Iron in Biological Materials.

By G. J. TRUTER, Section of Biochemistry and Nutrition, Onderstepoort.

SINCE the time of Ossian (1837) cited by Woods and Mellon (1941) the thiocyanate iron method has been tackled by many investigators. However, a review of the literature reveals a most variable, and in some cases contradictory mass of information on the use of the thiocyanate reagent in the determination of iron.

The thiocyanate method is based on the fact that ferric iron and an alkali thiocyanate give a red colour in an acid solution. At present there are still conflicting hypotheses as to the nature of the coloured complex. Schlesinger and Van Valkenburgh (1931) found that an anion $\text{Fe}(\text{CNS})_3$ is responsible for the colour. This concept could in no way be substantiated by Bent and French (1941) who did find definite proof for the colour to be due to a cation FeCNS^+ .

The purpose of the work described in this paper is to throw more light on: (i) The influence of various ions, especially calcium, phosphorus, and copper on the red complex. (ii) The acid best suited, its optimum concentration, and whether absolute control of concentration is necessary. (iii) The influence of temperature on colour intensity. (iv) The effect of the thiocyanate present, and (v) an expedient in the form of an oxidant to obviate colour fading.

HYDROGEN PEROXIDE AS A MEANS OF ARRESTING THE REDUCTION OF FERRIC TO FERROUS IRON.

One of the main objections to the thiocyanate-iron colour system, is the fact that the colour fades in aqueous solution. This fading of the colour is due to the reduction of ferric to ferrous iron by the thiocyanate; a fact which can be easily established by heating the aqueous solution of the coloured salt, thereby accelerating the reduction process, until the colour disappears. If an oxidizing agent is added the colour reappears.

Hydrogen peroxide may be used to excellent advantage as an oxidizing agent to ensure that the iron remains as the ferric ion. Peters *et al* (1939) report that hydrogen peroxide develops a yellow colour with thiocyanate.

The lowest concentration of hydrogen peroxide at which a yellow colour has been noted by the author is 0.12 per cent. Satisfactory results were obtained with a concentration of 0.004 per cent. H_2O_2 (see table 3).

THE ACID BEST SUITED AND ITS OPTIMUM CONCENTRATION.

The test is carried out by some workers in hydrochloric acid solution [Elvehjem (1930), Farrar (1935)]; by others in sulphuric acid solution [Leeper (1930), Scott cited by Daniel and Harper (1934)], and in nitric acid solution [Winter (1931), Woods and Mellon (1941)].

During the present investigation it was found immaterial whether hydrochloric acid or sulphuric acid is used. The effect of nitric acid is variable. By making use of this acid errors of up to a few hundred per cent., depending upon the amount of acid used and other circumstances such as time of contact and temperature, may be made.

Walker (1925) found that ordinary nitric acid gave a red colour with thiocyanate. He further pointed out that the red colour was produced not by the nitric but by the nitrous acid it contained. This statement needs some elucidation. It is known that nitrous acid is the great offender, but although nitric acid does not produce an instantaneous colour with thiocyanate, it has been found that the oxidation-reduction reaction between nitric acid and thiocyanate (potassium thiocyanate was employed) might be "catalyzed" under certain conditions with the liberation of nitrogen dioxide which is also extracted by the amyl alcohol. If the concentration of the nitric acid is kept low, 0.25 N, no colour is obtained, but in such a case the concentration of the acid is too low to act as an efficient oxidizing agent for maintaining the iron in the ferric state.

As is evident from Tables 1 and 2, a relatively wide range of acid concentration is permissible. However, as will be pointed out later on, high concentrations of acid and thiocyanate are essential for efficient colour development in phosphate rich solutions. A concentration of 0.45 N hydrochloric or sulphuric acid, and of 0.4 N potassium thiocyanate in the presence of 0.0025 N hydrogen peroxide is optimal for ferric thiocyanate colour development.

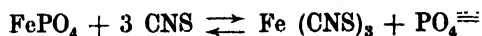
For many wet-ashed samples it has been found immaterial whether the aliquot was first neutralized with strong ammonium hydroxide, and then reacidified, or, whether the colour was developed directly in the sulphuric acid medium. Table 2 shows that a concentration of ammonium sulphate equivalent to 0.6 ml. of concentrated sulphuric acid can be tolerated in a solution free from other interfering substances. However, the total mass action of a high concentration of other substances especially phosphates already present in the testing solution together with the additional ammonium sulphate will cause a greater reduction of the ferric-thiocyanate colour potential. It is thus advisable to develop the colour directly in the solution as prepared from wet ashing.

THE EFFECT OF THE THIOCYANATE PRESENT.

According to the law of mass action it is logical to expect a higher concentration of thiocyanate to be accompanied by a corresponding intensification of the colour (see discussion).

In studying the relationship between colour intensity and thiocyanate concentration Peters and French (1941) came to the conclusion that the colour intensity increases linearly as the CNS/Fe ratio increases. The effect of increasing thiocyanate concentration on colour intensity is clearly presented in Table 1. The linear relationship between colour and CNS/Fe ratio referred to above, could however, not be confirmed. Due to this effect of the thiocyanate it is of the utmost importance to have its concentration the same for both standard and unknown.

As far as orthophosphates are concerned the effect of the thiocyanate present can clearly be seen from the following reaction.



This is the reason for advocating a relatively high concentration of thiocyanate for phosphate rich solutions in order to shift the equilibrium for the above reaction as much as possible to the right.

Apparatus.

A photo-electric colorimeter (the Leitz type was used in this work) pyrex ignition tubes, 8×1 in., and medium sized Kjeldahl flasks for greater samples.

Reagents.

(1) Standard iron solutions. Stock solution.

Any of the following procedures may be followed:—

(a) Dissolve 0.5000 gram of "analytical" iron wire in 20 per cent. sulphuric acid to which 3 ml. concentrated nitric acid have been added. Carefully evaporate until copious white fumes appear, cool, and transfer quantitatively to a 1 litre volumetric flask and dilute to volume 1 ml.=0.1 mg. of iron.

(b) Dissolve 0.7022 gram ferrous ammonium sulphate $[\text{FeSO}_4 (\text{NH}_4)_2 \text{SO}_4 \cdot 6 \text{H}_2\text{O}]$ in 100 ml. doubly distilled water. Add 5 ml. concentrated sulphuric acid. Warm slightly and titrate with 0.1 N KMnO_4 solution until just pink. Dilute to 1 litre.

1 ml.=0.1 mg. of iron.

This latter stock solution was made use of.

Working Standard.—Dilute 100 ml. of the stock solution to 1,000 ml; adding 10 ml. of a 0.1 per cent. hydrogen peroxide solution or its equivalent, prior to adjusting to volume.

1 ml.=0.01 mg. of ferric iron.

(2) A 50 per cent. potassium thiocyanate solution (50 gram per 100 ml. of solution).

(3) Reagent grade hydrochloric and sulphuric acids, concentrated.
Nitric acid, concentrated, redistilled.
Perchloric acid, 60 per cent, redistilled.

(4) Hydrogen peroxide; 0.1 per cent. solution in doubly distilled water, kept in a brown bottle, in a dark place, preferably in a refrigerator.

(5) Iso-amyl alcohol. Recovered amyl alcohol from residues is not satisfactory.

PREPARATION OF SAMPLE.

It is a familiar fact that dry ignition of biological materials for iron analysis yields erroneous results if special care is not taken. The wet combustion method has proved not only to be safe in the hands of an experienced worker, but advantageous in many respects. All pyro- or metaphosphates are, for instance, hydrolysed to the ortho form, thus doing away with a special hydrolysis during the sample preparation.

The Combustion Procedure.—1 ml. of blood (or .5 to 2 grams of tissue depending on the suspected iron content) is measured into a 8×1 in. pyrex tube. A glass bead, 2 ml. concentrated sulphuric acid, 3 ml. 60 per cent. perchloric acid, and a few drops of concentrated nitric acid (0.3 to 0.5 ml.) are added, and the material digested over a micro flame in a fume cupboard. When the initial, sometimes vigorous, oxidation reaction has subsided, nitric acid is added drop-wise until the oxidation is completed and the solution clarified. The final solution should be colourless or, at most, a greenish-yellow colour. Products of high fat content do not lend themselves so readily to complete oxidation. If the HNO_3 is unable to clarify the solution, the contents are allowed to cool slightly, a further 0.5 to 1 ml. HClO_4 added, and the heating continued. A few more drops of HNO_3 may be necessary to clarify the solution. The heating is continued until the appearance of characteristic dense white fumes of sulphur trioxide.

At this stage the solution may be expected to be free from perchloric and nitric acids. However, decomposition products of these acids are sometimes tenaciously retained by the concentrated sulphuric acid (e.g. nitrosylsulphuric acid), even after prolonged boiling. Hydrolysis with about 2 ml. of water, and subsequent boiling to white fumes effect the removal of these products. During the present investigation it was found sufficient to boil the diluted contents just for about one minute. When no evolution of gas takes place upon dilution boiling is unnecessary. Roberts *et al* (1940) are of the opinion that in the case of milk nitrosylsulphuric acid cannot be decomposed by the usual hydrolysis procedure. Refuge has to be taken to 30 per cent. hydrogen peroxide, a dropwise addition of 1 ml. to the warm sulphuric acid solution, completely decomposing the nitrosylsulphuric acid. The digest is allowed to cool, a further 10 ml. redistilled water are added, and quantitatively transferred to a 100 ml. volumetric flask.

Cool to room temperature and make up to volume.

PROCEDURE OF DETERMINATION.

After a test experiment has been conducted to determine the approximate iron content of the sample, transfer the chosen aliquots to pyrex tubes similar to those used for combustion, marked at 25 ml. Adjust the acid concentration if necessary, add 1 ml. of the 0.1 per cent. hydrogen peroxide solution and fill up to the 25 ml. mark with redistilled water. Add exactly 10 ml. isoamyl alcohol to be followed by 2 ml. of the 50 per cent. potassium thiocyanate solution. Close the tube with a stopper, previously extracted with 10 per cent. hydrochloric acid and shake for 30 seconds. Transfer the amyl alcohol phase to centrifuge tubes, and centrifuge for 5 minutes at 3,500 r.p.m. to get rid of water particles suspended in the alcohol extract. Obtain readings with filter 430, setting a reagent blank at 100 per cent transmission. The iron values are obtained from a standard curve. No unnecessary time should be allowed to elapse between the addition of the thiocyanate and the extraction of the red complex from the aqueous solution.

TABLE 1.

The effect of different concentrations of hydrochloric acid and potassium thiocyanate on the Ferric Thiocyanate complex.

Concentrated HCl.	50 % KCNS.	Transmission.	Concentrated HCl.	50 % KCNS.	Transmission.
ML.	ML.	Per Cent.	ML.	ML.	Per Cent.
0.2	0.5	54.5	0.5	6.0	44.8
0.2	1.0	49.0	1.0	0.5	59.0
0.2	2.0	48.0	1.0	1.0	49.5
0.2	3.0	47.5	1.0	2.0	48.0
0.2	4.0	47.0	1.0	3.0	45.0
0.2	6.0	47.5	1.0	4.0	44.8
0.5	0.5	57.5	2.0	0.5	60.5
0.5	1.0	49.0	2.0	1.0	50.0
0.5	2.0	48.0	2.0	2.0	48.6
0.5	3.0	45.0	3.0	2.0	51.5
0.5	4.0	45.0	4.0	2.0	54.0

TABLE 2.

The effect of sulphuric acid or a sulphate on the red complex.

Tube No.	(NH ₄) ₂ SO ₄ * (Grams.)	HCl (Concentrated ML.)	Transmission, Per Cent.	Tube No.	H ₂ SO ₄ Concentrated ML.	Transmission, Per Cent.
1	0.486	1.0	48.2	8	0.2	48.0
2	0.729	1.0	48.0	9	0.3	48.3
3	1.215	1.0	48.3	10	0.5	48.0
4	1.458	1.0	48.0	11	0.6	49.0
5	1.944	1.0	49.0	12	0.8	49.5
6	2.430	1.0	49.0	13	1.0	50.0
7	3.645	1.0	50.0	14	1.5	50.0

* The amount of ammonium sulphate introduced is the equivalent of the sulphuric acid in the second last column.

TABLE 3.

The stabilizing influence of hydrogen peroxide on the ferric thiocyanate complex.

Time Elapsed before Shaken Out of the Aqueous Phase. (Min.)	TRANSMISSION.	
	No Hydrogen Peroxide Added.	Hydrogen Peroxide Added. (1 ml. of 0.1 % solution.)
	Per Cent.	Per Cent.
0.....	48.0	48.0
5.....	48.2	48.2
10.....	48.0	48.0
15.....	48.3	48.2
25.....	48.5	48.3
35.....	49.0	48.2
45.....	50.5	49.0
65.....	53.5	48.0

NOTES ON THE THIOCYANATE IRON REACTION.

The apparent stability of the colour for the first 15 to 20 minutes in the tubes without added peroxide, is accounted for by a relatively strong oxidizing agent present in Mercks extra pure iso-amyl alcohol. This has been verified, and constitutes an added convenience of considerable value. However, not the least coloration is obtained between this alcohol and thiocyanate even after standing for a few days. When amyl alcohol free from any oxidizing agent, for instance, those of the British Drug Houses (B.D.H.) was used, a marked reduction in colour intensity took place within the first 5 minutes, and proceeded more or less linearly with the time at a constant temperature. The reduction potential is a function of both the time and the temperature.

THE EFFECT OF TEMPERATURE ON COLOUR INTENSITY.

A point of interest to note here is that the colour of the thiocyanate complex is a little more intense at a temperature of 15° C. and lower, than at higher temperatures; so much so that a difference of 1 per cent. to 3 per cent. transmission might be obtained. This phenomenon has probably nothing to do with a possible contraction of the amyl alcohol, and hence a corresponding intensification of the colour. Although the temperature of the alcohol was allowed to rise after the extraction of the colour, the transmission still remained the same. A further investigation into the secrets and properties of the coloured complex may perhaps yield an answer for this behaviour.

TABLE 4.

Tube No.	Temperature of Test Solution.	Transmission.	Tube No.	Temperature of Test Solution.	Transmission.
	°C.	Per Cent.		°C.	Per Cent.
1.....	30	48.0	5.....	20	48.0
2.....	28	48.1	6.....	18	48.0
3.....	26	48.0	7.....	15	47.0
4.....	24	48.2	8.....	5	45.2

The red complex seemed to be fairly stable in the amyl alcohol. In no cases were increases in transmissions noted of samples read within 30 minutes after extraction of the colour provided the temperature was not higher than 27° C. On a few occasions not the slightest difference in transmission was obtained even at the end of 24 hours. However, it is absolutely essential not to make use of recovered alcohol that has been used before. Iso-amyl alcohol recovered from residues is liable to errors varying from 10 to 20 per cent. within the first 5 minutes after extraction.

PHOSPHATE INTERFERENCE.

Various statements occur in the literature as to the interference caused by phosphates in the formation of the colour of ferric thiocyanate. It is evident from Table 5 that the effect of orthophosphates has been exaggerated. Results concordant with the control were obtained with aliquots containing up to the equivalent of 87 mg. of phosphorus. Leeper (*loc. cit.*) on the other hand gave 100 mg. P_2O_5 (i.e. 43.7 mg. P) as the safe limit. Walker (*loc. cit.*) quoted 50 mg. P_2O_5 (i.e. 21.8 mg. P) as the limit. A lower tolerance for

phosphate can without exception be accounted for by a lower concentration of acid and thiocyanate. It is alleged by Winter (*loc. cit.*) that with a proper acidity and a sufficient excess of thiocyanate no difficulty is encountered from phosphates.

TABLE 5.
Effect of orthophosphate on the recovery of iron.

Sample No.	Iron Present.	Phosphorus Added.*	Per Cent. Iron Recovered.
	Mg.	Mg.	
1.....	0.01	17.4	100.0
2.....	.01	52.5	100.0
3.....	.01	87.0	100.0
4.....	.025	17.4	100.0
5.....	.025	52.2	99.2
6.....	.025	87.0	92.3
7.....	0.03	17.4	99.5
8.....	.03	52.2	96.0
9.....	.03	87.0	90.5

* The phosphorus was added as $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ with an iron content of 0.1 mg. per cent.

It is quite clear that the lower the concentration of iron the greater the tolerance for phosphate. Farrar (*loc. cit.*) presented results to indicate that "concentrations of orthophosphate of 70 mg. per cent. or greater cause fading of the ferric thiocyanate color with amyl alcohol, however, permits easy colorimetric estimation of 0.01 mg. of iron". Thus it is always advisable especially where a high concentration of phosphate is suspected to carry out the determination with an aliquot rather low in iron content.

Both pyro- and metaphosphates prevent the reaction between thiocyanate and iron. [Woods and Mellon (*loc. cit.*)]. They are, however, quantitatively hydrolysed and changed to orthophosphates by the strong acids during wet ashing. This can be seen from the data of Table 6 which are very similar to those of Table 5.

TABLE 6.

Recovery of iron from pyrophosphate solutions treated with the wet ash procedure.

Sample No.	Iron Present.	Phosphorus Added.*	Per Cent. Iron Recovered.
	Mg.	Mg.	
1.....	.01	13.9	100.0
2.....	.01	41.6	99.8
3.....	.01	69.4	100.0
4.....	.025	41.6	99.5
5.....	.025	69.4	94.5
6.....	.03	41.6	96.6

* Phosphorus was added as $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$.

Calcium has been reported by Daniel and Harper (1934) to interfere with the quantitative recovery of iron if present in excess of 10 mg. per 100 ml. of an hydrochloric acid solution. Recovery data presented in Table 7 indicate that calcium interference is negligible when the proposed method is applied.

TABLE 7.

*The joint effect of calcium and phosphorus or calcium alone on the recovery of Iron.**

Sample.	Kind of Acid Used.	Calcium† Added.	Phosphorus‡ Added.	Per Cent Iron Recovered.
		Mg.	Mg.	
1.....	HCl	20	None	100.0
2.....	HCl	40	None	99.8
3.....	HCl	200	None	100.3
4.....	HCl	400	None	100.5
5.....	HCl	20	17.4	99.8
6.....	HCl	20	52.2	98.8
7.....	H ₂ SO ₄	20	17.4	99.8
8.....	H ₂ SO ₄	20	52.2	98.5
9.....	H ₂ SO ₄	80	None	99.9
10.....	H ₂ SO ₄	200	None	100.2

* 0.025 mg. of iron added to all solutions.

† Calcium added as CaCO₃.

‡ Phosphorus added as Na₂HPO₄·2H₂O.

With samples of high calcium content the possibility does exist that iron may be occluded by the fine precipitate of CaSO₄. No marked occlusion occurred for the few cases studied in Table 7. A well-defined precipitate of CaSO₄ has not once been found for the tissues analysed in Table 9, except for bone in which case it is advisable to conduct a dry ignition with subsequent hydrolysis in hydrochloric acid.

One would expect the thiocyanate method to have its limitations in the presence of many other substances. Thus, according to Walker (loc. cit.) no or unsatisfactory colour development is obtained in the presence of salts of silver, mercury, cobalt or large amounts of copper. Salts of these elements occur normally in such low concentrations in biological materials that the determination of their influence on the recovery of iron was deemed unnecessary. However, in cases of enzootic icterus, a jaundiced condition of the liver, exceptionally large amounts of copper accumulates in the bodily organs especially the liver. Values varying between 60 mg. per cent. and 600 mg. per cent. for dry sheep liver have been found at this Institute. In view of work that is in progress in this connection it was considered necessary to obtain recovery data on iron in the presence of various concentrations of copper and on iron added to these organs.

The apparently higher recoveries for iron is accounted for by the fact that copper reacts with thiocyanate giving a yellowish colour which is also extracted by the amyl alcohol. The choice of a suitable aliquot for the iron determination is thus governed by the copper content of the sample. In cases of enzootic icterus studied, copper has been found to constitute no trouble in

the determination of iron, especially as the iron content of the organs was found to exhibit the tendency to be much higher than under normal circumstances.

TABLE 8.
Copper interference on Iron Recovery.

Tube No.	Iron Present.	Copper Added.*	Transmission.	Per Cent. Iron Recovered.
	Mg.	Mg.	Mg.	
1.....	·025	0·0	48·0	100·0
2.....	·025	·005	48·0	100·0
3.....	·025	·01	48·1	99·8
4.....	·025	·02	48·1	99·8
5.....	·025	·03	48·0	100·0
6.....	·025	·04	47·5	101·0
7.....	·025	·05	47·0	102·0
8.....	·025	·10	46·0	104·0

* Copper added from an "electrolytic" copper standard solution.

TABLE 9.
Analyses of Healthy Animal Tissues (Bovine).

Tissue.	Copper Content.	Iron Content.	Iron Added.	Total Iron Calculated.	Total Iron Found.
	P.p.m.	P.p.m.	P.p.m.	P.p.m.	P.p.m.
Blood.....	1·2	510	100	610	594
Brain.....	7·5	233	50	283	278
Heart.....	16·0	378	50	428	420
Liver.....	90·0	555	100	655	654
Liver.....	280·0	354	100	454	458
Kidney.....	12·5	482	100	582	560
Spleen.....	6·8	11,500	200	11,700	12,000
Liver*.....	2100·0	3,300	100	3,400	3,300
Kidney*.....	230·0	230	150	380	372
Adrenal.....	11·7	315	50	365	366
Muscle.....	3·5	210	150	360	365
Thyroid.....	1·8	120	200	320	315
Bone marrow.....	0·85	23	100	123	120

* Sheep organs. A case of enzootic icterus.

DISCUSSION.

Except for a few modifications the procedure of digestion given in this paper is essentially the same as that given by Eden and Green (1940). Better results have repeatedly been obtained with more sulphuric acid than with the one ml. advocated by these authors. Furthermore the addition of a few drops of concentrated nitric acid prior to starting combustion is a decided advantage and very effective in shortening the time of digestion. The addition of 2 ml. sulphuric acid and 3 ml. perchloric acid, advocated to start

oxidation can be varied according to circumstances, depending on the final acid requirement or the bulk of the sample. The introduction of too much perchloric acid, however, constitutes a fair amount of danger and care should be taken not to exceed the ratio of $3\text{HClO}_4:1\text{H}_2\text{SO}_4$.

The use of an organic solvent such as iso-amyl alcohol is a satisfactory means of extracting the coloured complex, the colour intensity of which is dependent upon the degree of dissociation (K), from a solution with a relatively high di-electric constant.

If we consider the reaction:—



where m and n are the charges on the complex, then the equilibrium reaction will be given by:—

$$K = \frac{(\text{Fe}^{+++})^m (\text{CNS}^-)^n}{\text{Fe}_m (\text{CNS})_n}$$

It is clear that the concentration of undissociated $\text{Fe}_m (\text{CNS})_n$ molecules, and thus the colour intensity of the solution is inversely proportional to the value of K.

The proposed method has proved entirely satisfactory when applied to a wide variety of animal tissues (Table 9). For aliquots containing up to 25 micrograms of iron (30 micrograms are the limit for a suitable reading) recoveries of better than 0.5 microgram are possible. The greatest loss of iron usually occurs during the digestion procedure and great care should be exercised to avoid spluttering of the contents.

SUMMARY AND CONCLUSIONS.

1. A modified procedure of the thiocyanate method for the micro-determination of iron in biological materials is described.
2. Evidence is presented to show its efficiency in the presence of moderate quantities of copper and relatively large concentrations of phosphate and calcium.
3. Hydrochloric or sulphuric acid is preferable to nitric acid, which should rather be avoided.
4. For colour stability it is essential to avoid recovered iso-amyl alcohol previously used for either iron or copper determinations.
5. A detailed description of a wet combustion procedure is given.
6. Some data on the iron and copper contents of tissues are given.
7. It can be concluded that the proposed method not only satisfies the need for a time-saving and simple procedure, but also conforms to the requirements for accuracy and reliability, if the necessary precautionary measures are taken.

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The Effect of Diet and Body Condition on the Heat Regulating System of the Merino Sheep.

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INTRODUCTION.

IN all biological investigation the rôle exerted by intercurrent factors is a matter demanding constant recognition and attention. This is also clearly seen in bioclimatological work on animals where the reaction to climatic forces is constantly being influenced by the physiological state of the individual and the extent of its adaptation. In this connection it is to be expected that in addition to the rôle played by species and age the nutritional status of an animal would exert itself as a dominant factor in determining the body response to external environmental conditions including those of climate. From a survey of the literature there is, however, little to indicate in how far the plane of nutrition may affect the reaction of an animal to climatic conditions. In view of this fact a series of experiments was undertaken on merino sheep in which the physiological response of separate groups comprised of well-fed and poorly-fed animals was measured under identical conditions of exposure either to heat or to cold.

EXPERIMENT No. 1.

Procedure.

The first experiment was carried out during the winter months of June and July. Sixteen merino sheep (castrated males) of similar stature and age (4 to 6 tooth) were selected, eight as being in good condition and eight in poor condition. The animals were placed in individual feeding pens under a galvanized roof but open at the sides. The better-conditioned sheep were fed 350 gm. yellow maize daily in addition to lucerne hay *ad lib.*, while the poorer sheep received poor quality grass hay *ad lib.* only. All the animals were given 3 gm. NaCl per day and water was unrestricted. The average daily consumption of these diets was (a) well-fed group—350 gm. maize and 790 gm. lucerne hay. (b) Poorly-fed group—600 gm. grass hay.

After an initial period of one month on the above diets, one half of the well-fed group was given grass hay only and half of the poorly-fed sheep were put on to the maize and lucerne ration. This resulted, therefore, in the following groups:—

1. Lucerne and maize throughout.
2. Lucerne and maize followed by grass hay.
3. Grass hay throughout.
4. Grass hay followed by lucerne and maize.

The Effect of the Diets on Body Weight.

TABLE 1.

Group.	Days in Experiment.	0	30	50
1	Diet.....	—	Good	Good.
	Av. body weight in lb.....	104	108	107
2	Diet.....	—	Good	Poor.
	Av. body weight in lb.....	110	109	97
3	Diet.....	—	Poor	Poor.
	Av. body weight in lb.....	78	67	57
4	Diet.....	—	Poor	Good.
	Av. body weight in lb.....	83	75	82

From the above table it will be seen that the diet of maize and lucerne was sufficient to maintain the weight of the good-conditioned sheep and to cause an increase in that of the poor sheep. The hay ration, on the other hand, was inadequate and caused a steady drop in the body weight of all the sheep to which it was fed.

The Effect of Diet on Rectal Temperature.

After the sheep had been on the experimental rations for 21 days, their rectal temperatures were recorded at 8 a.m. and 3 p.m. daily. This was done while the animals were in the feeding pens protected from the direct rays of the sun by the roof.

For the sake of simplicity only groups 1 and 3, i.e., those which received the good and poor rations throughout the experiment respectively, will be dealt with first.

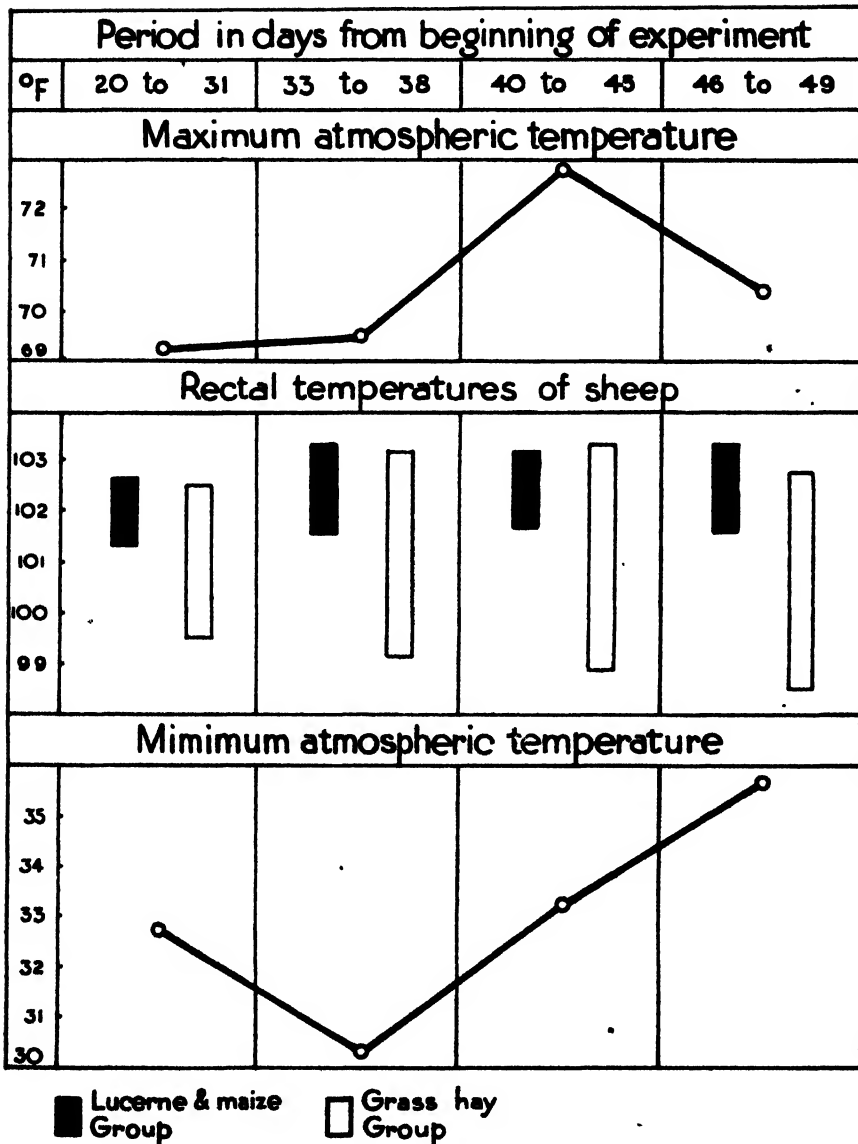
Graph I depicts the average rectal temperatures at 8 a.m. and 3 p.m. respectively of groups 1 and 3 over different periods during the experiment. The bottom end of the columns represent the figures for 8 a.m. and the top end those for 3 p.m. The length of the column, therefore, depicts the range between these two readings. The average maximum and minimum atmospheric temperatures recorded during the same periods are given above and below respectively.

It will be noted from this graph that the rectal temperatures of the poorly-fed sheep (unshaded columns) taken at 8 a.m. were considerably lower than those of their well-fed mates (black columns) and that this difference became steadily accentuated as time passed and the poorly-fed animals became progressively more emaciated. Furthermore this decline in the rectal temperature in the early morning was independent of the slight rise in minimum atmospheric temperatures encountered during the latter part of the experiment and must be attributed either to the poor diet or to the consequent drop in body condition.

The Effect of Change of Diet on Rectal Temperature.

As already indicated, 31 days after the commencement of the experiment, half of the sheep on the maize and lucerne ration was given grass hay only, while half of those previously on the poor ration were fed maize and lucerne. These groups are designated 2 and 4 respectively.

GRAPH I.

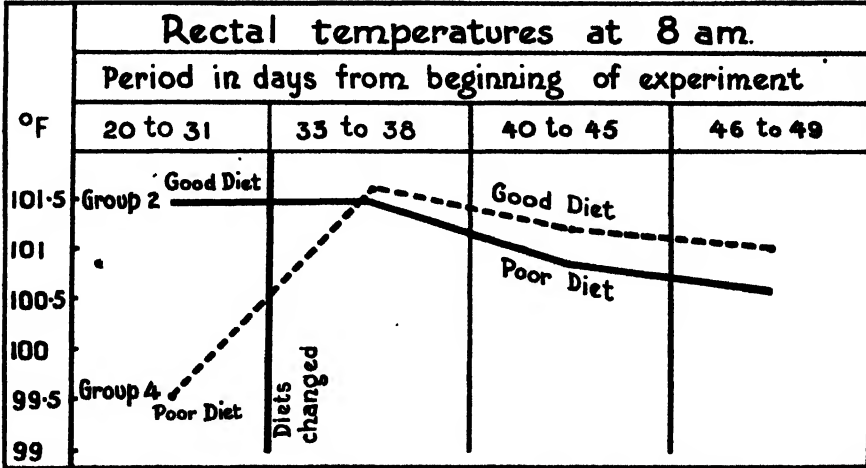


The effects of these changes in diet on the rectal temperatures at 8 a.m. are shown in Graph II. As the figures for the 3 p.m. readings remained unchanged they are not included in the graph. It will be seen that the better diet caused an immediate rise in the rectal temperatures of the poor sheep while the inadequate diet caused a more delayed and less marked decline in that of the good-conditioned animals. These findings indicate that the food intake of sheep has a direct effect on their ability to maintain the body

EFFECT OF DIET AND CONDITION ON HEAT REGULATING SYSTEM OF SHEEP.

temperature during exposure to cold. The changing over of the diets of these two groups also refutes the legitimate objection that the original grouping was done on body condition and not at random.

GRAPH 11.



EXPERIMENT No. 2.

Procedure.

In view of the above findings a second experiment was commenced on the 5th of August. A further 16 sheep of similar type to those used in the first experiment but all in medium condition were placed in the feeding pens as before. The same rations as previously described were used, four sheep being given lucerne and maize, and twelve grass hay.

The animals were placed in their pens and fed daily at 8 a.m. and again at 4 p.m., the feeding troughs remaining in the pens continuously. At 8 a.m. immediately prior to the morning feed, the respiration rates and rectal temperatures were recorded. At noon the sheep were put out on a concrete floor in the open sun without any food but with water available. At 3 p.m. the respiration rates and rectal temperatures were again recorded. In this way the reactions of the sheep were tested to both cold overnight and to heat during the day.

TABLE 2.

Ration.	Average Body Weight (lb.).	
	Initial.	After 30 Days.
Maize and Lucerne.....	70	74
Grass Hay.....	62	56

The effect of the different diets on the body weights of the animals was similar to that in the first experiment as will be seen from the figures in Table 2.

The Effect of Diet on Rectal Temperature.

The procedure described above was continued for the first 30 days of the experiment, the respiratory rates and rectal temperatures being recorded from the ninth day onwards. The findings are shown in graph III which will be found on page 325 of the text*. The period now to be discussed, i.e., from the 9th to the 30th day is designated "Period 1". The same system of plotting the rectal temperatures as used in graph I is again employed, that is the 8 a.m. readings are plotted below and the 3 p.m. readings above. The *unbroken columns* represent data from the *well-fed* sheep and the *broken columns* from the *poorly-fed* animals. The letter S denotes that a statistically significant difference at 5 per cent. level was found between the rectal temperatures of the two groups on that particular occasion.

It will be seen from graph III that the morning rectal temperatures of the poorly-fed sheep were again consistently lower than those of the well-fed animals, this difference ranging from 0.5 to 2.0° F. Further there was a tendency for the poorly-fed sheep to be more directly affected by variations in the atmospheric temperature.

On the other hand it will be noted that at 3 p.m., i.e., after remaining for three hours in the sun, the undernourished sheep, on warm days, showed an average rectal temperature up to 0.8° F. higher than that of the sheep on a good ration. On the two days (16th and 30th) on which the atmospheric temperature did not exceed 75° F., the thin sheep showed an average rectal temperature below that of the well-fed group. These findings afford evidence therefore that under-nourishment rendered the sheep more thermo-labile with the extremes of environmental temperature reflected to an exaggerated degree in their body temperature.

Period 2.

Thirty days after the experiment was started, four of the sheep receiving grass hay were given the ration of maize and lucerne, thus introducing a third group (indicated by dotted columns on the graph). The time during which these three groups were maintained is designated Period 2 in Graph III. In other respects the experiment was continued as before.

It will be seen from the graph that the relations between the rectal temperatures of the original groups (1 and 2) continued as before. The behaviour of the new group (3) will now be discussed.

Twenty-four hours after the change in diet (32nd day) the average morning rectal temperature of these sheep had risen by 1.4° F. and showed a statistically significant difference (1 per cent. level) from the temperatures of the sheep still receiving the grass hay only. The change from a poor to an adequate diet, therefore, again caused an immediate rise in the rectal temperature after exposure to cold weather (30° F.).

It will be seen, however, that the temperatures of group 3 taken at 3 p.m. did not for some considerable time differ materially from those of the under-fed sheep, the first significant difference occurring 21 days after the change

* The reader is asked to follow the subsequent discussions on Graph III.

in the diet, i.e., on the 51st day of the experiment. The appearance of this reaction was probably delayed by the cold weather encountered between the 43rd and 49th days of the experiment as shown by the maximal temperatures for that period. In this connection it is of interest to note that during this cold spell the afternoon temperatures of the underfed sheep were markedly depressed and were actually below those of both the groups on an adequate ration.

Period 3.

After the experiment had been running for 45 days the animals in group 1 (maize and lucerne ration throughout) were removed and the work continued with groups 2 and 3. As will be seen from the graph the animals receiving the adequate ration (group 3) gradually regained their ability to control the rise in body temperature when exposed to heat, the rectal temperature of this group at 3 p.m. becoming significantly lower than that of the poor animals.

The Effect of the Diet on the Respiratory Rate.

Before proceeding to a discussion of the respiratory rates of the three groups it should be emphasized that in sheep as in other ruminant animals respiration assumes special significance owing to the fact that a considerable amount of carbon dioxide normally eliminated from the lungs is derived not only from the tissue metabolism but through absorption from the digestive tract in which active fermentation of carbohydrates is a characteristic feature. Moreover, in the absence of an active sweating mechanism coupled with a thick wool covering, body temperature in merino sheep is largely controlled through changes in respiration.

Respiration at 8 a.m.

It will be seen from graph III that, at 8 a.m., the sheep on the poor diet (broken lines) showed respiratory rates approximately one half (± 20 per min.) of the well-nourished animals (± 40 per min.). As the question of heat elimination need not be considered in the temperatures encountered at this time of the day, this depression in the speed of respiration can be attributed to a decreased tissue metabolism and to a lesser absorption of carbon dioxide from the alimentary tract. It will, however, be seen that rate of respiration of the well-fed group appears to have been more definitely influenced by the level of the minimum atmospheric temperature recorded the previous night, than was the case with the underfed group.

Furthermore it will be noted that when the diet of group 3 (dotted line) was changed from grass hay to maize and lucerne (see Period 2 on graph III) these animals showed a gradual rise in their respiratory rate at 8 a.m. until it was equal to that of group 1 on the 10th day after the change in ration. This rise in respiratory rate did not coincide with the rise in body temperature the latter occurring much more promptly.

The Respiratory Rate at 3 p.m.

The undernourished sheep showed a slower respiratory rate (± 40 per min.) than their well-fed mates after three hours' exposure in the sun (80 to 160 per min.). This absence of panting might be considered as being the cause of the higher rectal temperatures simultaneously recorded, but as will

be seen in Period 2, the change in the ration of group 3 caused a prompt rise in their rate of breathing without a corresponding drop in body temperature (from 31 to 34 days). This indicates that in sheep changed from a poor to a good diet the consequent alteration in digestion and metabolism immediately causes a change in the relationship of respiratory rate to body temperature.

EXPERIMENT No. 3.

In order to verify these findings in an acute experiment, representative animals from groups 1 and 2 were tested in a hot box. This apparatus consisted of a thermostatically controlled, electrically heated cabinet into which the animal was placed with the head protruding. The temperature inside the box was maintained at between 105° and 108° F. and each animal was kept in it for a period of one hour. The results of such an experiment are shown in Table 3.

TABLE 3.

Sheep No.	Diet.	Body Weight (lb.).	Prior to entering Box.		After 1 Hr. in Box.		Difference.	
			Resp.	Temp.	Resp.	Temp.	Resp.	Temp.
1	Good....	71	30	103.3	132	104.0	102	0.7
2	Good....	75	36	102.8	106	103.7	70	0.9
13	Poor....	45	20	100.4	20	102.1	Nil	1.7
14	Poor....	50	22	102.9	24	104.6	Nil	1.7

These results afford striking evidence that undernourishment and poverty cause a suppression of the normal panting reflex and marked thermo-lability in experimental sheep.

DISCUSSION.

It has been clearly shown that both general body condition and diet have a marked influence on the heat regulating system of the merino sheep. The effect on the resistance to cold and to heat will be dealt with separately.

(a) *The Maintenance of Body Temperature in a Cold Environment.*

The subnormal rectal temperatures shown by the thin, undernourished sheep after exposure to cold can be attributed to a lack of heat-producing material in the body. One of the objects of this experiment was to ascertain in how far digestion and appetite were affected by a poor diet in conjunction with exposure to cold. It was found, however, that appetite was well maintained while ruminal movements and defaecation were unimpaired and no secondary complications such as nutritional oedema or pneumonia were encountered. In other words the poorly-fed sheep appeared thin but healthy, the only demonstrable effects of exposure to cold being the low rectal temperature and slow respiratory rate. This indicates therefore that, in the merino sheep, the control of body temperature is the first of the vital functions to be impaired by undernourishment, and not digestion itself as had been suspected when these experiments were started.

The immediate rise in body temperature in the early morning shown by poor sheep after being given an adequate diet must be explained as being due to the availability of heat-producing material and to the specific dynamic action of the food. The promptness of the response is of great interest and also of practical value when dealing with emaciated stock in severe winter conditions as it indicates that even poor sheep may respond immediately to a good diet.

On the other hand it was shown in Experiment 1 that good-conditioned sheep when suddenly placed on an inadequate ration were able to utilize their body reserves for the maintenance of body temperature for some considerable time. Under the conditions of the experiment this period was approximately 10 days.

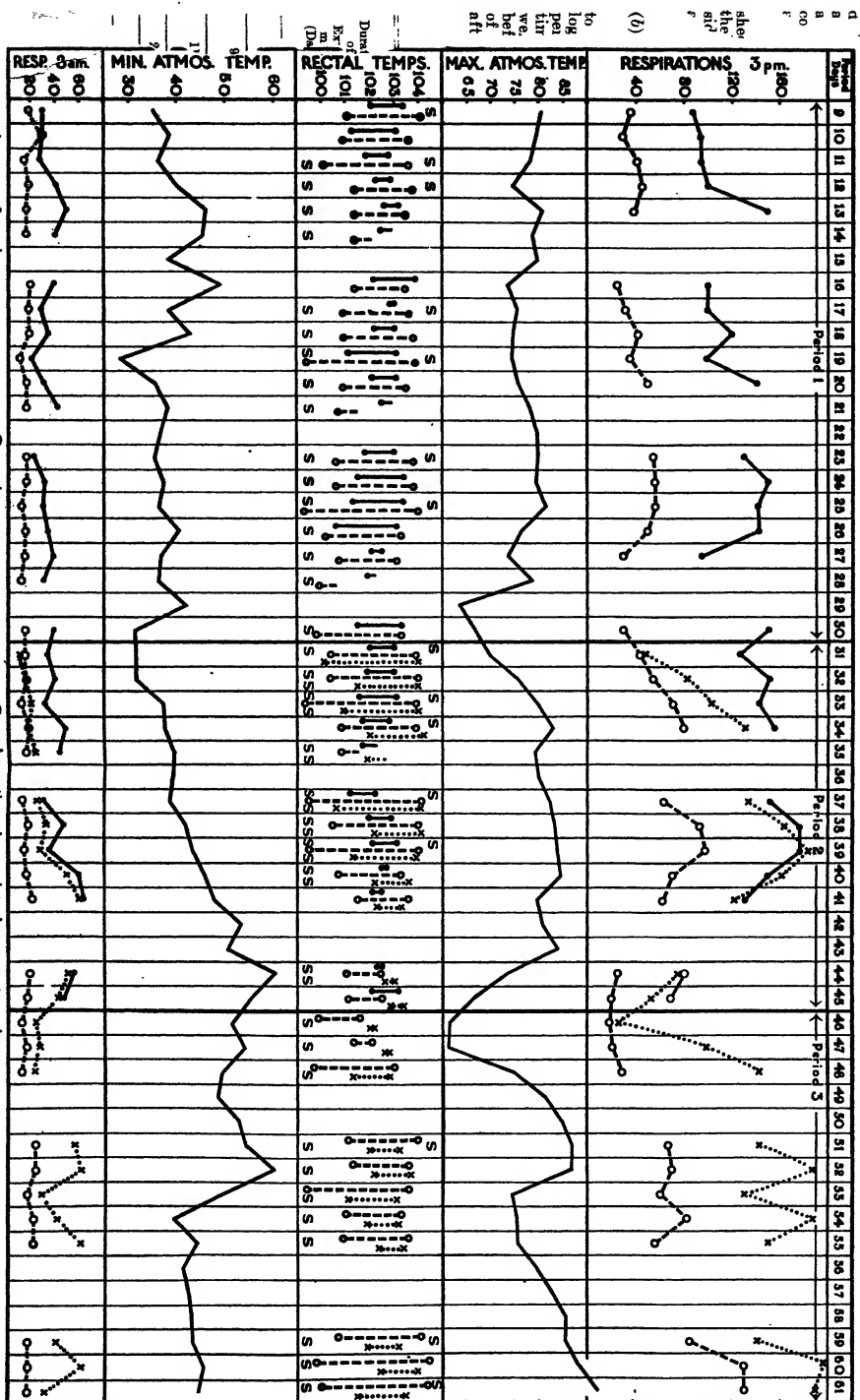
(b) The Control of Body Temperature in a Hot Environment.

The inability of the poor sheep to control body temperature when exposed to heat depends upon the general body condition probably through the physiological state of the central heat-regulating mechanism together with the peripheral vasomotor control and not on the calorific value of the diet at the time of the test. This statement is based on the fact that when thin animals were placed on a good diet (Exp. 2, Group 3) there was an interval of 21 days before there was any significant difference between the rectal temperatures of these sheep and those of their counterparts still receiving the poor ration, after exposure to the sun. Table 4 summarizes this aspect of the results.

TABLE 4.

Duration of Experiment (Days).	Average Body Weight.			Remarks. (See Graph III).
	Gr. 1 (Well Fed).	Gr. 2 (Poor Diet).	Gr. 3 (Poor Diet).	
0	70	63	—	—
9	—	—	—	Body temp. Group 2 significantly higher than Group 1 at 3 p.m.
11	70	57	—	Body temp. Group 2 significantly lower than Group 1 at 8 a.m.
25	74	57	53	—
30	—	—	(Well Fed)	Ration Group 3 changed.
31	—	—	—	Body temp. Group 3 significantly higher than Group 2 at 8 a.m.
33	76	54	51	—
51	75	51	61	Body temp. Group 3 significantly lower than Group 2 at 3 p.m.

GRAPH III.



It will be seen from the above that the poorly-fed sheep (group 2) showed an excessive rise in body temperature when exposed to the sun at about the time that their average body weight fell below 60 lb. Conversely well-fed animals (group 3) displayed an increased power to prevent a rise in rectal temperature when their average body weight had increased to a similar figure. It would, therefore, appear that under the conditions encountered in this experiment and for the class of sheep used, 60 lb. can be looked upon as a critical body weight, under which a breakdown in the resistance to heat may be expected.

SUMMARY.

1. It is clearly shown that the diet and general body condition of sheep markedly influenced their heat-regulating mechanism.

2. The maintenance of the body temperature of thin sheep in a cold environment was found to be closely associated with the calorific value of the diet at the time of exposure.

3. Moderately conditioned sheep maintained their body temperature when exposed to cold for a period of 10 days after being placed on an inadequate ration.

4. The control of temperature in a hot environment was found to depend largely on general body condition.

5. When the sheep were exposed to heat the following observations were made:—

(a) Thin sheep on a poor diet showed a suppressed panting reflex and an excessive rise in body temperature.

(b) Thin sheep on a good diet showed a normal panting reflex but also an excessive rise in rectal temperature. This anomaly cannot as yet be fully explained. The ability to control the body temperature returned when the general body condition improved.

6. Contrary to expectation, continued poor feeding of sheep in conjunction with repeated exposure to cold failed to cause any clinical disturbance either in digestion or in the normal appetite of such animals. The effect of this treatment on the heat regulating system of the body, however, was both clear and pronounced.

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Meat Studies No. 1.—Post-natal Growth and Development of Muscle, as Exemplified by the Gastrocnemius and Psoas Muscles of the Rabbit.

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*(Accepted as a thesis in partial fulfilment of the requirements for the degree of Doctor of Veterinary Science in the University of Pretoria, November, 1944.)**

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* In preparing for publication minor changes have been made in the text. An additional chapter on relative growth by D. van der Reyden, Section of Statistics, has been included.

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CHAPTER I.—INTRODUCTION.

(a) INTRODUCTION.

HITHERTO the subject of meat production was approached mainly from the feeding side, and generally stopped at the digestion of the foodstuff and the body weight of the animal.

This traditional approach was broken by Hammond (1932). He made observations on the final product—meat—and worked backwards to determine the conditions and factors which affect its formation. Macroscopic methods mainly, were applied in making a general survey of the scientific principles involved in the production of meat, from the physiological, anatomical and practical points of view.

Scattered throughout the literature are isolated references to microscopical meat studies, on small numbers of animals, of different species and varying uniformity. Due to the diversity of conditions under which the observations were often made, and in many cases too, due to a lack of accurate definition of procedure, these studies cannot easily be co-ordinated.

A wide field of investigation lies open to the worker who approaches the problem of meat quality from the histological point of view. Definition of the quantitative character of muscle, in terms of measurable biological entities such as muscle bundle and muscle fibre, constitutes a primary requisite for such an investigation. In addition, the qualitative changes occurring in meat must be considered in relation to the variations in the morphology of muscle.

Such a microscopic biological study will establish a basis for studying meat in the various domestic animals. It will facilitate evaluation of various factors which affect its formation. Furthermore, that elusive character, meat quality, may be brought a stage nearer to precise determination when considered in terms of such study.

Accordingly, this work is devoted to a study of the morphological changes of muscle and its component units, during growth and development. The object is a general survey of the principles involved in muscle growth, particularly as it occurs within the individual muscle. It is hoped that these observations may suggest profitable lines of experimental work, dealing with development of muscle and meat quality.

Although it would be of advantage to commence investigations on the domestic animals used for meat production, such observations would be costly and time-consuming. Preliminary observations on an animal species completing its life cycle in a short while yield information at less expense and in a shorter period of time. Such information may be of value in establishing various factors concerning growth of muscle and its development.

Small laboratory animals live under different conditions from the usual meat animals. Moreover, the general principles of growth may not be identical in small and large domestic animals. Nevertheless, the information obtained may serve a useful purpose, by making it easier to plan meat investigations.

Preliminary observations indicated that the rabbit was more suitable than the other laboratory animals for the purpose of this study. Hence rabbit muscle was utilised for this work.

(b) OBJECT OF WORK.

The contractile properties of voluntary muscle have been investigated almost exclusively in cold blooded animals such as the frog, mainly because such muscle may be isolated and kept alive for a considerable time. Study has largely been confined to a few muscles such as *Gastrocnemius*, *Soleus*, and *Sartorius*. The physiology of warmblooded mammalian muscle is, to a large extent, interpreted in terms of the experimental behaviour of such frog muscle. This is not without difficulty. It is hardly surprising when the wide range of mammalian muscle, of varying architecture and function, is taken into consideration. Moreover, the work has been concerned with physiology (the nature of muscular contraction, its chemistry, and its efficiency), rather than morphology as such. Obviously there is still a wide field open for experimental investigation.

On the other hand, the long series of researches by Hammond (1932), and his co-workers Pålsson (1939-40), Verges (1939a, 1939b), and McMeekan (1940-41), represents „a return to the practice of the older days when animal physiology was not yet divorced from morphology”. These authors have dealt with the differential growth of constituent parts of the body in terms of muscle, fat, and bone, with the object of clarifying the biological problems involved in meat production. Muscle has been considered in terms of its proportional development in the different parts of the animal body. As the economic value of meat depends primarily on the proportion of edible meat to inedible parts of the carcass, these workers have utilised weight as a basis for their investigations.

However, growth and development of muscle mass must ultimately depend on growth and development initiated in the micro-structure of the muscle. Muscle is conceived as a network of connective tissue, binding together a mass of fibres which form the greater part of a muscle. Morphological change of these muscle fibres must largely determine the change in morphology of the gross muscle, as well as its change in weight.

In the present work, the morphology of muscle has been studied, with the object of deciding how this changes during growth and development. This is necessarily the first step. The next step is to determine what growth and developmental changes occur in the individual muscle fibre, and whether they account for the coincident change in size and shape of the muscle mass. Special emphasis will be laid on the relation between the growth made during successive periods, with the object of deciding whether further research on these lines is likely to prove profitable.

On account of the nature of the investigation, statistical treatment of the data collected is essential in order to obtain reliable quantitative results. However, the laborious character of the microscopic measurement in work of this nature places severe limitations on the extent of work which can be undertaken. This is likely to limit the value of the results by reason of the restricted scope of the investigation.

Hammond (1932) showed, that if muscles are arranged in different anatomical groups, growth follows well-defined gradients. However, individual muscles within the different groups "vary in their rate of growth and overlap in many places those of other groups". It follows that the behaviour of the muscle group as a whole cannot be accurately assessed from any single muscle within the group.

It can be inferred that similar difficulties are inherent in the present study, if attention is confined to a limited number of muscles. There is the danger fallacious properties may be attributed to musculature in general. Furthermore, although a localised description of isolated muscles may show up minor variations it is not likely to afford any idea of the general laws of growth. Justification of the method lies in the fact that the present investigation is only a preliminary step to decide whether morphological analysis of muscle growth is likely to prove a profitable avenue of meat research. Subsequently the study may be expanded to include muscular tissue throughout the animal body, in order to establish more closely the relationship between muscle growth and development, and muscle type and structure.

Morphological study of muscle growth may appear remote from the basic problem in mind, namely meat investigation. It is to be emphasised that knowledge regarding the structural composition of muscle affords a ready means of comparison of meat, not only from different muscles within the same carcass, but also from different carcasses of varying grade and quality.

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Finally, I have great pleasure in expressing gratitude to my wife for her valuable services in the preparation of the manuscript. Besides typing a large portion of the work she also assisted in innumerable other ways.

CHAPTER II.—REVIEW OF LITERATURE.

For convenience the literature will be discussed under headings corresponding with those employed in the treatment of the experimental data. First, however, it is appropriate at this stage to consider briefly growth and relative growth.

By means of his autocatalytic theory of growth Robertson (1923) holds that master reactions regulate growth cycles, represented by peaks in the growth curve. However, Robb (1929) discredits this theory, as the cycles may be explained by natal and juvenile growth retardations, inevitably associated with the disturbance of birth, and of endocrine re-organisation at a later stage of life. Snell (1929) points out an inherent defect in the theory that growth rate is controlled by autocatalytic processes. MacDowell, Gates, and MacDowell (1930) also do not favour this interpretation. Hammond (1932), and Walton and Hammond (1938) disagree with the autocatalytic theory as they show that the growth curve is dependent on the food supply; thus, "these peaks at regular times in the life of an animal are due to the nutritive conditions usually existing at these times".

Recently analysis of growth has become increasingly directed towards the mathematical generalisation of experimental data. General formulae have been devised for describing the growth of the organism as a whole. Although these theoretical expressions are useful for comparison and tabulation, there is danger in attempting to define the fundamental growth process itself from empirical formulae.

In order to understand the growth of an organism it is essential to analyse the changes in form of the organism. Huxley (1924, 1932), and Huxley and Teissier (1936), formulated a law of simple allometry which is applicable over long periods of the animal's life, after completion of the stage of histological differentiation. When the growth of a part is considered in relation to the rest of the body, the relative rate of growth of the part and of the body remains constant. Huxley's equation expresses this law of relative growth by means of a formula $y = bx^a$, where y equals the

part, x the whole, b a constant representing the value of y when x equals 1, and a the equilibrium constant of the part. When a is greater or less than unity, the part is growing more or less rapidly respectively than the whole, that is, positive or negative allometry.

For a wide variety of data, this equation has been fitted to express the relation between a part and the whole, as the organism increases in size. cursory inspection of the literature discloses an amazing diversity of interests. [Pearsall (1927), Keys (1928), Robb (1929), Hersh (1931, 1934, 1938), Green and Fekete (1933), Needham (1932, 1934), Dawes and Huxley (1934), Lerner (1936), Rytand (1937-38), Gray and Newcombe (1938), Hamilton and Dewar (1938), Clark and Hersh (1939), Huggins (1940), Crozier (1940), Brody (1942), Kibler, Bergman and Turner (1943), Richards and Kavanagh (1943)]. The list is by no means complete, and it is intentionally selective in order to emphasise the general manner in which Huxley's equation has been applied.

There is no doubt that the formula affords a useful method of comparing curves of growth. Thus, instead of only being able to present a record of the differences of absolute size with age, the formula makes it possible to disclose more clearly the underlying morphological changes by showing alterations in the proportions of individual parts with increasing total size. By means of the equilibrium constant a , a measure is obtained of the relative increase or decrease of the part with increase in the absolute size of the organism.

On the other hand, doubts exist as to whether biologists have been sufficiently critical regarding the application of the formula, and whether the fit of their data to this equation is real or not. Discussion has taken place regarding the implications of Huxley's formula, both from the viewpoint of possible shortcomings as well as its undoubted advantages [Robb (1929), Davenport (1934), Bernstein (1934), Wilson (1934), Feldstein and Hersh (1935), Richards (1936), Lumer (1936, 1939), Kavanagh and Richards (1942), Lumer, Andersen and Hersh (1942)].

Huxley makes it clear that the allometric law is limited to the growth occurring after the processes of histo-differentiation have been completed. Thus, the varying proportions of newly-born reciprocal crosses between the large Shire horse and the small Shetland pony are determined by genetic influences acting before birth (Walton and Hammond, 1938). Although the proportions of the body subsequent to birth follow the law of allometry, nevertheless the constant a is approximately the same for the different crosses. Similarly, Pontecorvo (1929, 1938) finds that the equilibrium constant a is very nearly the same for widely differing breeds of cattle. Notwithstanding this similarity in their relative growth rate, the different breeds vary greatly in the adult condition. Apparently the initial absolute size of the parts and of the body at birth must play a big rôle in determining body size and proportions in later life.

Huxley also demonstrates the presence of certain growth gradients where the values of the relative growth rate a , obtained for a series of parts arranged in order along the organism, change systematically from one end of the series to the other. Similarly, growth centres are found from which the growth intensity grades downwards, by comparing the parts of an organism on the assumption that the values of a are constant within each portion. Although it is reasonable to assume that the equilibrium constant

will vary in a progressive manner from point to point within the limits of a single part, the analysis does not take account of this continuous variation. Such an analysis is, however, likely to yield a more accurate knowledge of the growth mechanism.

(a) GROWTH AND DEVELOPMENT OF THE RABBIT.

Post-natal growth has been extensively studied for most animal species. Reference to Brody, Ragsdale and Elting (1926), or Hammond (1932, 1940a) gives an idea of the extent of literature available.

Absolute weight increases slowly in the growing animal at first, then more rapidly. The live-weight curve for rabbits normally shows a steady rise gradually flattening with age as maturity is approached. About the period of sexual maturity it receives a temporary check. Later, an increase in weight is again noticeable in most cases and the animal subsequently becomes somewhat heavier, largely due to deposition of fat after the cessation of growth (Punnett and Bailey, 1918; Castle, 1922; Pease, 1928).

While the animal is growing, body conformation and shape are undergoing continuous change as a result of the different parts growing at different rates (Hammond, 1932; McMeekan, 1940-41). Jackson and Lowrey (1912-13) point out that, in the rat, the intensity of growth passes over the body like a wave, reaching the maximum first in the head and fore-limbs, and later passing backwards along the trunk to the abdominal portion and hind legs. Hammond (1940a) states: "In general, the wave of growth beginning at the head, spreads down the trunk, and secondary waves which start at the extremities of the limbs pass upwards; these all meet at the junction of the loin with the last rib, which area is the last part to develop. Such growth gradients also exist between different tissues in the body which develop in the following order—brain, bone, muscle and fat."

Rate of growth varies in different breeds of rabbits. Large breeds usually mature more slowly than small breeds, hence, in general, the small rabbit will attain its mature weight earlier than a large rabbit (Dunlop and Hammond, 1937). Although heavy weight is closely associated with slowness of maturity, Pease (1928) points out that many rabbits show conspicuous absence of this association. Data have been presented showing a maximum rate of growth about 30 days after birth (Murray, 1921), sixty days (Wilson, 1930), whereas Dunlop and Hammond's (1937) large strains "E" and "H" attained the maximum about 100 days compared with 40 days in their small strain "F". Robb (1929) finds a distinct tendency for two peaks in the lifetime of the animal, one about 40 days after birth and the other at about 100 days. These figures illustrate the differences inherent in different breeds.

It is hardly surprising that workers using different breeds in various parts of the world are not unanimous regarding the age at which the rabbit attains sexual maturity. Thus, Punnett and Bailey (1918) estimate puberty at 10 months in Polish and over 12 months in Flemish rabbits; Castle (1922) 6 to 7 months; Hammar (1932) 4 to 5 months; and Fangauf and Immenkamp (1938) 5 to 7 months.

Earlier workers regarded mature weight as the maximum weight attained during the first year of life. However, Pease (1928) points out that this arbitrary measure has no relation to the rate of growth of the rabbit. He uses instead, the "turning point", in his comparative growth

studies. This is defined as the point where the live-weight curve for the individual rabbit slackens off at the oncome of puberty. Pease states the live-weight curve gradually rises again after the turning point, then declines, and finally rises once more to adult weight at about 400 to 500 days.

There seems to be little doubt that age is not as important as weight in influencing the normal body changes and proportions, as the magnitude of one body part tends to be a specific function of the total body mass (Robb, 1929; Huxley, 1932). In the albino rat, Outhouse and Mendel (1933) describe a close correlation between increase in weight and length. They found so little relationship to age, that body dimensions and proportions were identical in animals of the same weight irrespective of their age. Size of muscle, and organ too, is dependent on body size of animal, not age (Moment, 1933). Dunlop and Hammond (1937) show that changes in the body proportion of the rabbit occur with weight rather than with age as such. For sheep too, weight classes rather than age classes at shows are suggested by Hirzel (1939), because the proportion of muscle within the sheep's body is influenced by increase in weight more than age. It follows, therefore, in planning comparative growth studies, live-weight rather than age must form the basis of comparison of normal animals. It is to be noted, however, where growth is suppressed by under-nourishment, the magnitude of an organ or system may vary markedly for any given body-weight according to the age of the animal and the general state of nutrition (Jackson, 1932).

In most species, the male appears to be slightly heavier than the female at birth; in cattle (Hulce and Nevens, 1917; Eckles, 1920); in sheep (Donald and McLean, 1935; Phillips and Dawson, 1937; Bonsma, 1939); in pigs (Carmichael and Rice 1920; Murray, 1934); in guinea-pigs, Haines (1931); and in rats (King, 1935; Murray, 1941). As a rule the male continues to be heavier than the female, so that in most mammals the adult male is larger and heavier than the female. However, Kopec (1924) reports the weight of the two sexes is not essentially different in newly-born rabbits. Furthermore, Punnett and Bailey (1918) find the buck is in no case markedly heavier than the doe at maturity. Although the average weight is approximately equal in some cases, yet the doe is often markedly heavier than the buck. In the larger races of rabbits, the male has a bigger frame and is consistently larger in all bone measurements, nevertheless the female puts on more flesh and surpasses the male in weight (Castle, 1922). Castle cites breed standards for the various large breeds, in which rabbit breeders regularly specify a larger weight for females than for males. MacDowell (1914) is of opinion that the growth subsequent to four months of age is greater in the doe than in the buck. Pease (1928) could find no difference in average weight of the two sexes, but bodyweight was nearly always more variable in does than in bucks. He suggests a heavier weight is prescribed for does by show standards, because the female sex is more variable. This greater variability in live-weight of females is confirmed by Dudley and Wilson (1943). In addition, these authors find that the average live-weight of females after puberty is greater than that of males. Wilson (1930), and Wilson and Morris (1932), observed noticeable differences in the composition of male and female flesh. In general, the musculature of does at 11 months to 24 months of age contains 4 to 6 per cent. more fat than for the buck.

It is not clear how much the growth of rabbits is affected by the seasons of the year. Under the conditions of Pease's (1928) experiment, growth rate is unaffected by the season of the year in which the rabbit is born, or in

which it reaches maturity. However, Wilson (1929) holds that Spring and early Summer are the most favourable periods for satisfactory growth. This is contradicted by Bertelli (1936), who shows rabbits born during Autumn obtain their complete development sooner than those born in Spring.

(b) GROWTH AND DEVELOPMENT OF MUSCLE.

1. Weight.

Jackson and Lowrey (1912-13) cite numerous authors regarding the relative weight of skeletal musculature in widely varying species. Thus, in most adult mammals, between 40 per cent. and 50 per cent. of the body-weight is composed of muscle. Among mammals, the smallest relative weights are found in the large animals, while the largest percentage of muscle is recorded in comparatively small animals [rabbit, 49·7 to 57·2 per cent., Weiske (1895); 49·4 to 56·8 per cent., Levine *et al* (1941).]

Hammar (1932) presents evidence that the musculature of the rabbit grows most rapidly, and has its period of greatest growth about puberty. He states muscle grows more than twice as much during the two months around puberty, as during the two months immediately preceding. Hence, the animal at puberty becomes muscularised to a striking degree.

Hammond (1932) and McMeekan (1940-41) show that, in the sheep and pig, bone makes its greatest growth in the early stages of life, followed by muscle at a later stage, while fat attains its maximum growth still later. In the rat, the skeletal musculature increases from a relative weight of 22·82 per cent. at one week old to 45·43 per cent. at one year of age, in sharp contrast to the skeleton which decreases from 18·47 per cent. to 10·91 per cent. (Jackson and Lowrey, 1912-13). In the fowl, the skeletal muscles increase from 21 or 22 per cent. at hatching to about 50 per cent. of the body weight in the adult, compared with a relative weight of the skeleton at hatching or slightly less than 16 per cent., afterwards decreasing to about 8 to 11 per cent. (Latimer, 1924). In the human, the relative weight of the skeleton remains practically unchanged from birth to maturity (17·69 and 17·60 per cent.), whereas voluntary muscle shows a marked increase from 24·80 per cent. in the newborn babe to 43·07 per cent. in the adult (Wilmer, 1940).

The increasing proportion of muscle with age is explained by Hammond (1932), by the greater rate of growth of muscle to bone in the different parts of the body, but also by the greater growth rate of the later maturing parts of the body which contain large proportions of muscle to bone (e.g. loin compared with head and limbs). Even after muscle has attained maximum development, there is an increase in inter- and intramuscular fat, which tends to increase the weight of muscle.

Hammond shows that weight of muscle, regarded as a measure, has a late period of maximum development, as it is an index of muscle and fat development. Length development is attained relatively early. Hence, because increasing muscle and fat development with age increase thickness of muscle, weight can also be considered as an indirect measure of muscle thickness.

Hammond's studies make it clear that muscle groups develop serially in a definite manner, corresponding to the differential growth gradients existing between the different parts of the body. Growth waves pass from lower to upper limb, from the cranium backward, and from the tail forward,

to meet in the lumbar vertebrae, so that muscle in the loin and pelvis makes the most growth post-natally. However, within each group of muscles the rate of growth of individual muscles varies greatly and overlapping occurs between groups. Hammond also shows how these normal age changes are emphasised by sex, breed, domestication, and fattening.

Hammond clearly indicates that if an individual muscle is to be taken as a sample of a carcass, it is advisable to select a muscle with a late rate of post-natal development. *M. Psoas major* has been studied as a physiological unit of musculature with a view to obtaining information on the musculature in general (Callow, 1935, 1936, 1937, 1938; Woodman, Evans, Callow and Wishart, 1936). This muscle has the advantage of relatively late development; moreover it can readily be removed without cutting the carcass. In the pig, McMeekan (1940-41) finds that the weight of the *Psoas* muscle has a significant correlation with the total weight of muscle in the carcass.

(2) *Length.*

By the time adult life is reached the long bones have achieved their maximum growth in length, the other tissues growing in proportion with the growth of the bones in length. Haines (1932) explains the growth in length of a muscle as following on the lengthening of the bones to which it is attached, in response to the traction set up within the muscle by the bone growth. This stretch, which the growing skeletal system places on the muscle, probably influences considerably the increasing strength of skeletal muscles in growing animals, as the period of greatest increase in strength coincides with the period of rapid increase in the length of the long bones (Knowlton and Hines, 1939). As muscle growth follows and is so closely dependent on bone growth, it is not out of place to digress for a moment to consider the growth of bone.

Hammond (1932) and McMeekan (1940-41) show that bone length reaches a maximum relatively early in life, before muscle development takes place. Hence, lengthening of any individual muscle must reach a maximum earlier than its growth in width and depth, which depend on the development of both muscular and fatty tissues.

With regard to the relative change in length of different muscles as the animal grows, the lengthening of muscle units in various parts of the body must be influenced by the well-defined differential growth relationship for individual bones, demonstrated by Hammond and McMeekan. In the heifer, Eckles and Swett (1918) report a greater degree of lengthening for the vertebral column than for the hind limb (117.3 to 66.3 per cent.). The same is true of the sheep. Hammond (1932) gives measurements for Suffolk rams, from which the following table has been calculated.

Relative length growth with age.

	Birth.	Three Months.	Five Months.	Four Years.
Lumbar vertebrae.....	100	305.6	330.0	427.8
Femur.....	100	314.8	247.9	309.9
Tibia.....	100	197.1	224.1	278.7
Cannon.....	100	176.5	197.9	213.6

Whereas the ruminant is born in a relatively mature condition with long legs to follow its dam, the rabbit is comparatively immature at birth. Hence, caution must be exercised in drawing an analogy between different species. However, for the pig, where the limb bones are not so well developed at birth compared with the sheep, the same tendency is shown by McMeekan (1940-41). The following table, compiled from his scale photographs of the lumbar vertebrae and femur, shows clearly that the lumbar vertebrae lengthen to a relatively greater degree than the femur.

Relative length growth with age.

	Birth.	Four Weeks.	Eight Weeks.	Twelve Weeks.	Sixteen Weeks.	Twenty Weeks.	Twenty-four Weeks.	Twenty-eight Weeks.
Lumbar vertebrae.....	100	154	231	292	339	423	431	477
Femur.....	100	154	217	253	287	329	345	370

It can be inferred that M. Psoas, which is closely adherent to the vertebral column, will show a similar difference in length growth, as compared with a muscle from the upper limb.

(3 and 4). *Width and depth.*

In general, width and thickness are late maturing body measurements (Bonsma, 1939). Latimer (1927, 1928) shows that, after puberty in the foal, there is no increase in the length of bone, but the bones become stouter and increase in weight. Similarly, in the rat, the adult bones are wider and thicker than at an earlier stage of development (Hammett, 1924). Also in the pig, thickness growth of bone is a late developing character compared with length of bone (McMeekan, 1940-41). It is shown by Hammond (1932), that in the sheep, growth in circumference of bone, i.e., thickness, persists after bone has ceased growing in length. This author demonstrates how the growth changes in muscle groups copy, in an exaggerated form, the coincident changes in the bones they surround. By analogy, it can be inferred muscle width and depth increase after length has become stabilised. As muscle width and thickness are an indirect measure of muscle and fat development, i.e., weight, which is a later maturing factor than length, this is to be expected.

Hammond (1936) observed the changes in shape of the Longissimus dorsi muscle, with increasing age of various species of domestic animals. He shows the medio-lateral axis (width) reaches maximum development earlier than the dorso-ventral axis (depth), so that depth of muscle becomes relatively greater in proportion to width of muscle, as an animal becomes older. McMeekan (1940-41) too, states that as the animal ages muscle width achieves stability, whereas depth increases at a still greater rate. A picture is presented of the muscle increasing equally in both width and thickness in the initial stages, later only by thickness growth in increasing amounts.

(c) GROWTH AND DEVELOPMENT OF MUSCLE BUNDLE.

1. *Technique of measurement.*

Satisfactory demarcation of the bundle unit is an immediate difficulty in the morphological study of muscle.

Many workers have utilised a variety of methods to measure length of muscle bundle. This will be considered in connection with the muscle fibre (pages 341-342). Accordingly, they are not mentioned at this stage.

Although bundle length may be measured fairly easily, thickness of bundle is not capable of rigid definition. The smallest units, the primary bundles, are formed by a number of closely adjoining parallel muscle fibres held together by interstitial connective tissue. Several primary bundles combine to form secondary bundles, secondary bundles combine to form tertiary bundles, etc. A vast network of connective tissues binds together these bundles to constitute the individual muscle.

Hammond and Appleton (1932) judged bundle thickness by eye, because of the technical difficulties and labour involved in actual measurement. Sections were cut across the grain, from samples taken from the middle of the muscle. These sections were then graded, according to the coarseness of the component bundles. Hammond and Appleton point out that sectioning may introduce artefacts, as the bundles tend to fall apart more easily in some muscles than in others. Apart from this fact, in some muscles there are large bundles which are sub-divided into a number of smaller bundles, whereas in other muscles the bundles are all small. These authors confirm Piersol's (1920) finding that in muscles of coarse texture each bundle includes a number of sub-bundles, whereas in muscles with fine texture the secondary bundles correspond with the fasciculi.

Brady (1937), and Satorius and Child (1938) obtained a measure of bundle thickness, by counting the number of fibres in 50 bundles from each muscle, and by measuring the diameter of 50 muscle fibres from each muscle. McMeekan (1940-41) counted the fibres in 20 bundles selected at random, as well as measuring 100 fibres in each muscle.

2. Thickness of muscle bundle (texture, "grain").

Texture is important mainly because coarse texture is associated with tough stringy meat [Hammond 1940(a), 1940(b), 1942]. However, Beard (1924) finds that "the inherent properties of the endomysium contribute to the toughness of meat more than does the size of the fibre". Although there is also a broad correlation between toughness of meat and its connective tissue content (Mitchell and Hamilton, 1927-28; Moran and Smith, 1929; Mackintosh *et al.*, 1936; Bate-Smith, 1942), observations by Hammond (Moran and Smith, 1929, page 42) show that the proportion of connective tissue to muscle substance is considerably higher in the tender meat of foetal lamb than in the tougher meat of an adult sheep. This finding is corroborated for the rat by Hines and Knowlton (1939). These authors calculate that the connective tissue decreases from 40 per cent. of the total muscle mass at 15 days to 15 per cent. at 90 days of age. Hirzel (1939) comes to the conclusion that "evidence on texture and connective tissue, their interrelation and the effect on toughness of meat is still scarce and inconclusive".

Muscle texture is dependent on the size of the muscle bundles, which again depends on the number and size of the fibres comprising the bundle. Hammond and Appleton (1932) cite many authorities regarding texture of meat. Different muscles vary in texture; for example, Moran and Smith (1929) arrange beef muscles in order of increasing coarseness and toughness, from the M. Psoas (fillet), to Longissimus dorsi (rib), Biceps femoris (top-side) and lastly Semimembranosus (silverside). Muscles are fine-grained at

birth, but corresponding with the degree of enlargement of the muscle fibres, so does texture become coarser as the animal becomes older. Hammond and Appleton (1932) are of opinion that where the fibres are small, texture does not coarsen with age as much as in large-fibred muscles. Apart from differences within the animal, species differences are also evident. In general, a large species (ox) has muscles with coarser texture than a small species (sheep). Hammond and his co-worker show that, within a species, similar differences are present between large breeds and the smaller breeds.

The niceties of gradation of texture largely remain to be worked out. The extremes are probably represented by bundles with small numbers of fine fibres, as opposed to bundles with large numbers of thick fibres. Theoretically, there is possible an enormous range of intermediate gradations and combinations—small numbers of thick fibres, large numbers of fine fibres, etc. Possibly, size of bundle as such, is less important than the coincident association of thick bands of connective tissue in coarsely grained muscle, such as has been observed by Hammond and Appleton (1932).

(d) GROWTH AND DEVELOPMENT OF MUSCLE FIBRE.

Cobb (1925), Needham (1926), Hines (1927), Denny-Brown (1929), and Hammond and Appleton (1932), have reviewed the literature dealing with the histology of muscle. Most of the original articles are not obtainable in this country. This is understandable, as Needham remarks on the fact that the field of muscle histology has been almost deserted since 1909, when attention became focussed on the chemistry of muscle.

1. *Length of fibre.*

Maximow and Bloom (1930) state that muscle fibres are entirely independent structures, of cylindrical or prismatic shape, gradually constricting towards the ends and terminating in fine points. Particularly at the union of muscle with tendon, the end of the fibre may appear rounded, notched, or provided with teeth-like projections. These authors estimate the length of striated muscle fibres may vary from 1 to 41 mm. In short muscles, the fibres may continue through the entire muscle. In the larger muscles, the fibres are usually shorter than the muscle itself, and one or both ends may lie free within the muscle.

Huber (1916-17), working with adult rabbit muscle, dissociated single fasciculi into their component fibres. He found, in muscles with relatively short fasciculi (not longer than 2.5 cm.), the fibres extend from tendon to tendon. In semi-pinnate, pinnate, or compound pinnate muscles, also where the distal and proximal tendons overlap, the respective fasciculi are much shorter than the muscle itself. No fibres longer than 2.5 cm. were seen in the longest fasciculi teased out. In other words Huber found no fibres reaching from end to end of any fasciculi longer than 2.5 cm. In longer fasciculi, the fibres had either one blunt tendon end and one filamentous intra-fascicular termination, or the fibres were spindle-shaped ending in hair-like processes within the fasciculus. It is noteworthy, in only two fasciculi of a number teased from the Gastrocnemius muscle, one single fibre was found which did not extend from tendon end to tendon end.

Lindhard (1929) measured Gastrocnemius fibres in the frog. He reports the fibre runs from one terminal tendon of the fasciculus to the other terminal tendon. It is interesting to observe differences in two species of frogs

examined. In *R. esculenta* fibres are bluntly conical, whereas in *R. temporaria* the fibres are irregularly cylindrical, arranged in pairs, a thick and a thin fibre alongside each other.

Denny-Brown (1929) says of the Gastrocnemius medialis muscle of the cat: "Careful dissection of the fresh muscle with a wet knife shows every fasciculus runs from aponeurosis to aponeurosis. It was further found . . . that in any particular fasciculus the fibres run from end to end of the fasciculus. . . . No fibre was found which did not reach from aponeurosis to aponeurosis. All fibres, thick and thin alike, found their way from end to end of the fasciculus."

Buchthal and Lindhard (1939) give an excellent review of work dealing with the anatomy of the striated muscle fibre. They establish certain general types of fibre. Thus, cylindrical or bluntly conical fibres are comparatively short. Long muscle fibres are flagelliform, or lanceolate, connected to the terminal tendons by the thick rounded end, while the tapering end is lost in the endomysium. On the average, fibres shorter than the bundle are more than half the length of the bundles. Thin fibre-ends overlap at varying points within the bundle. Varying numbers of elongated spindle-shaped fibres, with both ends terminating in the endomysium, furnish additional mechanical support.

Hammond and Appleton (1932) measured only thickness of fibre. They point out, however, the size of the muscle is determined mainly by the number or length of the fibres, rather than by their thickness.

As muscle fibres are often of considerable length it is difficult to measure their length under the microscope. Moreover, the fibres are intimately interwoven and overlapped by other fibres, so that it is almost impossible to measure their length without completely isolating the individual fibres. This process is so laborious, it is incapable of routine application. Length of fasciculus, however, is more easily determined. Where fibres pass from end to end of the fasciculus, this measurement affords an idea of the fibre length. From the evidence cited, it appears that the Gastrocnemius muscle falls within this category.

2. Diameter of fibre.

(i) Technique of measurement.

Various methods of isolating muscle fibres for measurement of the shape and the dimensions are reviewed by Buchthal and Lindhard (1939). These authors stress the difficulty in evaluating the comprehensive histological literature concerning the muscle fibre, because most observations have been made on fixed and stained fibres. Different methods have been applied for measuring muscle fibre diameter by various workers, and only in very few cases have attempts been made to examine living fibres.

Lindhard (1926) boiled the muscle *in situ* for two hours in water. After isolating individual fibres under the low-power binocular microscope, he measured uninjured fibres with the aid of an ocular micrometer.

Paff (1930) made camera lucida drawings, on squared graph paper, of transverse paraffin sections of skeletal muscles of the rat, guinea-pig, and cat. He computed the average area of muscle fibres, by counting the square millimetres enclosed by the drawn outlines of six hundred different fibres, making seventy-five measurements for each muscle.

Clark (1931) used stained celloidin cross-sections in order to count the total number of fibres in skeletal muscles of the cat. The sections were projected on bromide paper at a magnification of seventy-five to a hundred diameters, and the fibres in each photograph were counted.

Hammond and Appleton (1932) cut free-hand shavings from a formalin-fixed strip from the middle of each muscle, and teased out the shavings on a slide in a drop of dilute glycerin. Average diameter was calculated by measuring the cross-diameter of fifty fibres by means of an eye-piece micrometer. The diameters of fibres in the middle and at the end of the muscle, in ten different muscles, from four animals, averaged $40\cdot10\mu$ for the middles, and $42\cdot37\mu$ for the ends. In six out of the ten muscles the ends had the slightly thicker fibres. Hammond and Appleton conclude there may be slightly more small fibres than usual, found in measurements of cross-diameters of fibres taken from the middle of the muscle.

McMeekan (1940-41) employed essentially the same method. He stained the shavings with picric acid, and mounted them in Farrant's solution.

Robertson and Baker (1933) macerated slender strips of fresh muscle in twenty per cent. nitric acid for two to four days. The macerated muscle fibres were washed with distilled water, and mounted in glycerin. Average diameter was calculated from the measurement of two hundred fibres. In addition, fibre size was indirectly estimated, in transversely cut sections, by counting the number of muscle fibres in an area $0\cdot207$ sq. mm. Twenty-five cross-section areas of the muscle were counted in order to calculate the average number of fibres in a square.

Voss (1935) utilised two methods to measure size of fibre. In a study of the leg muscles of the frog, he used a planimeter to record the area of cross-section of the fibres at a magnification of six hundred diameters. In an extensive tabulation of muscles from the human, dog, sheep, and hedgehog, a different method was employed. Here Voss counted the number of fibres in a square millimeter of cross-section to obtain an estimate of fibre diameter.

Brady (1937) isolated fibres by micro-dissection. A filar micrometer was used to measure the diameter of fifty fibres. Satorius and Child (1938) followed Brady's technique. It is worthy of mention that fresh unfixed tissues can be examined in this way.

(ii) *Fibre diameter.*

Perusal of the literature makes it readily apparent that the causes of differences in size of muscle fibres are a matter for speculation rather than assertion.

Hammond and Appleton (1932) find that the size of dark and of clear fibres, occurring side by side in the same muscle, varies independently of the colour of the fibres. Average size of fibre varies from muscle to muscle, but there is a more or less constant difference between the relative size of fibres in the different muscles. Denny-Brown (1929) maintains that there is no histological criterion of the speed of contraction of a muscle fibre. Although redness is generally associated with slowness of contraction, this is only a chance association with many exceptions. There is no relation between histological features, such as thickness or thinness of individual fibres, redness or paleness, and the rapidity or slowness of contraction. Voss (1935)

tested the correlation between fibre size and delicacy of movement of the muscle. He believes the more delicate the motion of which a muscle is capable, the finer is the degree of sub-division of the contractile mass.

Hammond and Appleton (1932) are of opinion that the differences in growth and development of muscles are determined by the interplay between complex factors such as evolution, function, and rate and degree of post-natal growth. Muscles used mainly for movement have on the whole smaller fibres than those used for maintaining posture probably because the small size of the fibre facilitates quick respiratory exchange (small pale fibre). Generally speaking, in the evolution of a muscle increasing in the history of a species, there is an increase in the number of fibres rather than their size, in order to increase activity and function of the muscle. With regard to rate and degree of post-natal growth, the earlier differentiating muscles tend to increase in size of fibre alone, whereas the latter differentiating muscles tend to be developed in number of fibres as well as size of fibre. On the other hand, in the individual after birth increase in number of fibres is not possible. Consequently, with increase of muscle function, an hypertrophy of the fibres occurs, together with an extra supply of myoglobin to facilitate respiratory exchange (large red fibre).

Donaldson (1915) cites Morpurgo's (1898) data regarding the number of muscle fibres in *M. Radialis* of the albino rat, from which it would appear that the fibres have increased by twenty-three per cent. at fifteen days, as compared with the new-born animal. Thereafter, until 420 days of age, cell multiplication is insignificant. Schultz (1934) reports that the muscle fibres of the frog increase in number with increasing age. She finds this post-natal increase proceeds more rapidly in younger than in older frogs, and continues until the number of muscle fibres is doubled. However, Hammond and Appleton (1932) state muscle growth after birth is mainly due to increase in size of the muscle cell, although they were unable to determine precisely at which stage muscle cell formation ceases in the sheep. McMeekan (1940-41) is unable to detect any increase in the number of fibres per bundle, in pig muscles, from birth to twenty-four weeks of age. Eliot, Wiggington and Corbin (1943) find that the number of muscle fibres in *M. Soleus* of the rat is not influenced by age of the animals. Consensus of opinion seems to favour this point of view, that growth of muscle occurs by hyperplasia in pre-natal life, and by hypertrophy in post-natal life (MacCallum, 1898; Schiefferdecker, 1919). Hence, it is to be expected that fibre diameter increases as the animal becomes older.

Apart from this thickening with age, good nutrition also increases the size of the muscle fibre. Conversely, defective nutrition reduces fibre diameter. Thus, Robertson and Baker (1933) find that muscle fibres of full-fed yearling steers are greatest in diameter and rough-fed smallest, while fibres from half-fed steers are intermediate in size. Black *et al* (1931) show that muscle fibres from steers fed a supplementary ration are slightly larger than those from steers on grass alone. Primitive breeds of sheep kept under poor nutritive conditions—semi-wild Shetland 45.5μ —have smaller muscle fibres than a highly improved breed reared on high nutrition—Suffolk 49.2μ (Hammond and Appleton, 1932). Similarly, McMeekan (1940-41) observes that pigs reared on a high plane of nutrition until sixteen weeks, have fibres roughly fifty per cent larger than individuals of the same breed reared on low nutritive conditions (12.08μ – 8.52μ). Moreover, this difference in fibre diameter is closely related to differences in the weights of both pig and muscle.

Kremer (1930) indicates that the musculature acts as a food reservoir in the hibernating frog. In consequence, the striated muscle is altered as this reserve is used. Voss (1937) maintains that starvation decreases fibre thickness in the muscles of the frog. Greene (1912), in an extremely interesting study, observes that the king salmon stores large quantities of fat in the muscular tissues, during its life in the ocean. It ceases to take food when it enters the fresh waters of the rivers in the journey to the spawning ground. Fat is gradually removed from the muscle during the migration period, so that it has almost disappeared when the fish has reached the spawning stage. Verne (1938) reports a marked decrease in the lipids in muscle fibre during fasting. Bell (1909) and Bullard (1916) describe lipoidal granules in muscle fibres, which are increased by feeding and reduced by starvation. Denny-Brown (1929) shows an increased granulation in the muscle of fattened cats, whereas the granulation seems to vanish in emaciated muscle.

As regards sex differences in size of muscle fibre, Eliot, Wiggington and Corbin (1943) observed no difference in size of fibre in *M. Soleus* of male and female rats. However, Hammond and Appleton (1932) report that the ram has larger fibres than the ewe, and wethers have fibres intermediate in size. Mehner (1938) states the muscle fibres, from *M. Gracilis* and *M. Sartorius* of chickens, are larger in the male. On the other hand, Brady (1937) and Sartorius and Child (1938) find that cows have significantly larger fibres than steers. It must be remarked that their experimental material comprised six Hereford-Shorthorn yearling steers and seven mature Holstein cows. As both age and breed are known to influence fibre diameter, it is unfair to attribute this difference to sex alone.

The effect of breed differences have been studied by Hammond and Appleton (1932). These authors show that the muscle fibres are larger in an improved breed of sheep than those of an unimproved breed. They believe there has also been an increase in the number of fibres in each muscle in the improved breed. They cite Malsburg (1911) to the effect that the heavier breeds of farm animals have larger fibres than the lighter breeds. Mehner (1938) confirms this finding. In a study of twelve races and crosses of ninety chickens, he finds the distinct racial differences in diameter of muscle fibre are almost parallel to the racial differences in body size. Mehner is of opinion the variations in size of the muscle fibres are almost enough by themselves to account for the differences in body size.

The comprehensive literature dealing with the effect of exercise on muscle has been reviewed by Steinhaus (1933). Various aspects of the problem have been investigated by Eliot, Wiggington and Corbin (1943), Fischer (1940), Bruman and Jenny (1936), Petré (1936), Petré *et al* (1936), Frey (1936), Rein *et al* (1935), Donaldson *et al* (1932, 1933), Donaldson [1935 (a), 1935(b)], Vannotti and Mageday (1934), Thörner (1930, 1934) Vannotti and Pfister (1933), and Regnault (1927). Consensus of opinion indicates that increased exercise produces increased vascularisation and hypertrophy of muscle. Steinhaus cites Siebert (1928), who states exercises of speed, strength, effort, induce hypertrophy of skeletal muscle, whereas exercises of endurance leave the body muscles unchanged in size. Morpurgo (1897), attributes hypertrophy to true enlargement of existing fibres solely due to formation of an increased amount of sarcoplasm. Thus, there is "no change in fibre length nor in the number of nuclei, nor the number or size of the fibrilli in the muscle cell."

(iii) *Colour or structure of muscle fibre.*

Colour of meat in relation to breed, condition, age, sex, feeding, management, exercise, and storage, is discussed by Hirzel (1939). In the higher mammals all muscles with few exceptions are red, but differences exist in the degree of redness between different muscles, also under different environmental conditions. For example, it has been found that the thigh muscles of the sheep are paler than the leg muscles. Moreover, the redness of these muscles increases with age and activity (Hammond and Appleton, 1932; Griffiths, Vickery, and Holmes, 1932). This redness is due to the haemoglobin content (myoglobin) of the muscle (Kühne, 1865; Whipple, 1926).

Millikan (1939) states: "Muscle haemoglobin is generally found in large quantities in those muscles requiring slow repetitive activity of considerable force." Hammond (1942) deduces the fatigue-resisting function of myoglobin, from the dark red colour of muscles of game animals living an active life, such as the hare, grouse, deer. These animals have darker coloured muscles than the domesticated rabbit, fowl and sheep. Within the same animal, colour differences may be explained on a like basis. For example, in the leg of the sheep, the muscle Extensor pedis which functions continually in maintaining posture is dark red in colour (Hammond and Appleton, 1932 (p. 497). In the rabbit, Roberts (1916) believes the red muscles play a prominent part in maintaining posture and fixing joints (M. Soleus, M. Crureus, deep head of M. Triceps).

Mention has been made of the extensive use of frog muscle for physiological investigation of muscle contractility. Although this muscle is pale and unpigmented, histological study reveals the presence of granular (sarco-plasmic, protoplasm-rich) and clear fibres (aplasmic, protoplasm-poor). Earlier workers attempted to homologise these two types of muscle fibre with the red and white muscles of birds and mammals.

Early workers studied especially the red and white muscles in the rabbit. They were inclined to homologise these two types with the histologically different dark and clear muscle fibres. Morphological differentiation was based chiefly on the relatively greater amount of sarcoplasm in the dark fibre, also the fact that the nuclei are not always found immediately beneath the sarcolemma as in the clear fibre. Hines (1927) cites Schaffer (1893), who reported also that the clear fibres in man contain small myofibrils arranged rather regularly, whereas in the granular fibres the fibrils are large and the arrangement without order.

Although striated muscle of higher vertebrates is red in colour, both dark and clear fibres are present so that few muscles are exclusively "red" or "white" in their make-up. Hammond and Appleton (1932), in a macroscopic examination of the leg muscles of the sheep, find all shades of colour between red and white linking up the extremes. Microscopical examination of these muscles showed that the proportion of dark, clear, and intermediate fibres varies according to the colour of the muscle, but intermediate fibres are numerous in practically all muscles.

Contradictory evidence is presented regarding age changes in the colour of muscle fibres. Denny-Brown (1929) states that the fibres of the pale muscle of the new-born kitten appear dark in cross-section due to the presence of numerous granules of some complex lipoidal substance. At fourteen days a proportion of fibres are clear and by a continuation of the process the

muscle ultimately becomes a mixture of dark and clear fibres. On the other hand, Hammond and Appleton (1932) find only clear fibres in the muscles of the newly born lamb. Later, in the five month old sheep, the majority of fibres are clear and intermediate in colour. In the adult sheep at twenty-two months, the full colour of the dark fibres has developed. The muscle fibres are on the whole darker than those of the younger animal, with a corresponding increasingly marked contrast in colour of dark and clear fibres. It is to be noted, in spite of the pale colour of the muscles in the lamb at birth, the fibres are structurally dark, with scattered nuclei and much sarcoplasm. McMeekan (1940-41) notes the presence of fat globules within certain muscle fibres of the newly born pig. These globules gradually decrease with age and cannot be detected after sixteen weeks. No specific mention is made, but presumably little alteration in colour of muscle fibres occurs up to twenty-eight weeks of age, as the muscle itself remains almost colourless throughout.

Owing to the confused state of knowledge, Cobb (1925) emphasises the need for more work before accepting physiological interpretations concerning red and white muscle. He suggests redness is not an essential feature. For example, colour varies in the same species (rabbit, hare) and genus (sedentary person, athlete). Cobb is of opinion histological criteria should rather be employed for differentiation of muscles i.e. primitive small fibres with centrally placed nuclei, as opposed to the more highly developed type with larger fibres, less sarcoplasm, and peripherally placed nuclei.

Lebedeva (1930) disregards colour differences. He considers muscles in the rabbit and cat fall into two classes. Dynamic muscles consisting of long parallel fibres have the typical structure of "white" muscle. Static muscles consisting of short fibres exhibit mostly a typical structure of "red" muscle.

Hammond and Appleton (1932) too, are inclined to the view that colour is not necessarily related to the structure of the fibre. They suspect "the differences in structure described in muscle fibres are the result of the degree of specialisation during development . . . differentiation rather than increase in size".

Hammond and Appleton cite Lewis and Stohr (1913) to the effect that enlargement of fully formed fibres takes place by increase of sarcoplasm. Similarly, Steinhaus (1933) quotes evidence to indicate hypertrophy is attributable to an increased amount of sarcoplasm. It follows therefore, that the histological appearance will be altered as a result of muscle hypertrophy, owing to the formation of an increased amount of sarcoplasm between the fibrilli of the fibre. Quite apart from this quantitative improvement of trained muscle, it may be expected that there will be a qualitative improvement by the formation of an increased amount of myoglobin in the hypertrophied muscle, with consequent reddening of the muscle. In fact, Bloor and Snider [1934(a), 1934(b)] show that additional muscular function results not only in a large increase of myoglobin, but in an increased phospholipid content as well. It is clear additional experimental evidence is required, of the effect on the micro-structure of muscle fibre of hypertrophied muscle, of these so closely associated responses to activity.

CHAPTER III.—PLAN OF INVESTIGATION.**(a) ANIMALS.**

Albino rabbits were purchased from four different local breeders prior to February, 1940 [July-August, 1938, 51; November, 1938, 46; March, 1939, 30; January, 1940, 19].

A small number of the outstanding animals from each batch was selected for breeding purposes by the officer in charge of the small animal establishment. Of the remainder, the majority were sacrificed for experimental requirements at this Institute.

In-breeding of the progeny of the original breeding stock was applied after January, 1940, to satisfy the local demand for experimental rabbits. All the animals used for this investigation were derived from the original stock, the earliest used in the experiment being bred in August, 1940, the last in November, 1943.

(b) HOUSING, MANAGEMENT, AND FEEDING.

Throughout the period of these investigations, the housing and management of the rabbits were unchanged.

From the time of weaning, each rabbit lived alone in a separate outdoor run communicating with a concrete hutch. The accompanying photos (Plates I and II) adequately describe the accommodation. However, dimensions are stated for the sake of completeness. A hinged iron roof covers the concrete hutch, which is 2 feet 6 inches long, 16 inches wide and 2 feet 6 inches high. Openings 6 inches wide, across the top of the front and back walls facilitate ventilation, and an opening at the bottom of the front wall allows entry into the run. This run, 6 feet long and 20 inches wide, also made of concrete, is enclosed by wire mesh. Attendants obtain access to the run by lifting the hinged wire mesh cover of the run. For drinking purposes, water was always available in a shallow trough outside in the run, whereas inside the hutch, a plentiful supply of clean veld hay was maintained for bedding. Throughout the year the small animal establishment is sheltered by the surrounding hedges. In summer particularly, shade is provided by poplar trees planted along the rows of rabbit houses. During the period of this experiment the rabbits were free of sickness. Except at parturition the mortality was nil, adequate testimony to their living conditions.

Freshly cut green foodstuff—lucerne or barley—was given at 8 to 8.30 a.m. each morning. In general, lucerne was supplied during the winter months, and barley in summer, but it largely depended upon what crop was available at the time. A liberal supply of the dry mash usually fed to rabbits at this Institute was placed in the food-pan at 2.30 p.m. daily, except in rainy weather when no dry ration was supplied. At 6.30 a.m. the following morning the remains were discarded. At this time the run was cleaned out, and the food-pan was washed and left outside the run until next feeding time.

This mash was composed as follows:—

Oats	65 per cent.
Wheat bran	20 per cent.
Linseed meal	12 per cent.
Bonemeal	2 per cent.
Lime	0.5 per cent.
Salt	0.5 per cent.

Because feeding of bran to animals was prohibited after June 1941 as a war measure, the mash comprised the following materials from that date:

Yellow maize meal—5 parts	} 60 per cent.
Crushed maize—1 part	
Crushed oats—2 parts	} 12 per cent.
Rolled oats—1 part	
Linseed meal	10 per cent.
Lucerne meal	10 per cent.
Meat meal	5 per cent.
Bonemeal	2 per cent.
Salt	0.5 per cent.
Lime	0.5 per cent.

Breeding does and bucks were housed essentially as described, except that the hutch and run had twice the floor space. When the doe was on heat, she was taken to the buck's hutch and removed after two "falls". Records were kept of the date young rabbits were born, their sex, the number in the litter, and the nest was inspected daily for some while after parturition.

Weaning of the young rabbits was carried out at seven to eight weeks. Sex was determined at this stage, and the buck rabbits were removed to their new homes, after weighing them. Identity of each rabbit was secured by tattooing a serial number in the right ear, by means of tattooing forceps.

For a variety of reasons, the young rabbits were at first weighed only after weaning. Later it became the practice to start weighing one day after birth, and to make daily weighings until seven weeks of age. Subsequent to this, weighings were carried out at weekly intervals until the rabbits were killed for the purpose of this experiment.

Weighing was always performed at the same time of the day, before the rabbits received their green food at 8 to 8.30 a.m., and the same day of the week—Saturday—was utilised for the weekly weighing. Circumstances with regard to food and time of day were thus as nearly uniform as possible.

It was rarely necessary to abandon weighing on account of rain, but on such occasions the weekly weighing was carried out on Sunday or Monday. If this was not possible, weighing was postponed until the following Saturday. In such cases, the average growth was obtained by dividing the total amount gained by two.

(c) NATURE AND SCOPE OF THE EXPERIMENT.

A complete study of post-natal growth and development of muscle should embrace observations from birth to death. Considerable time would elapse before the completion of such work, as senile material would only be available maybe six years after the birth of animals destined for that purpose. Furthermore, the limited facilities available for rearing rabbits (twenty hutches only) would severely limit the number of experimental animals.

Preliminary observations established the maximum weight of the strain of albino rabbits used as more or less 3,000 gm. Animals could be regarded as adult when they attained this weight. It was decided to investigate the

muscle growth accruing as a result of an arbitrary live-weight increase of 600 gm., commencing with the newly-born rabbit. Thus, observations were made on muscle taken from newly-born rabbits, at 600 gm. live-weight, and at 1,200, 1,800, 2,400 and 3,000 gm. live-weight. In addition, rabbits were kept for a period of six months after they first attained 3,000 gm. body weight, in order to determine changes as the animals matured.

When the data from these animals were analysed, it became evident that additional observations were desirable between birth and 600 gm. live-weight, in respect of certain muscle characters. Accordingly five additional groups were taken at 100 gm. live-weight, 150 gm., 220 gm., 320 gm., and 480 gm.

In growth studies, the most desirable data consist of measurements taken at comparable stages on a series of individuals followed throughout their period of development. This is obviously impossible in a study of this nature, where the animals must be killed in order to obtain the data. It must be assumed the older rabbits are what those killed at the earlier ages would have become at a later stage of their lives.

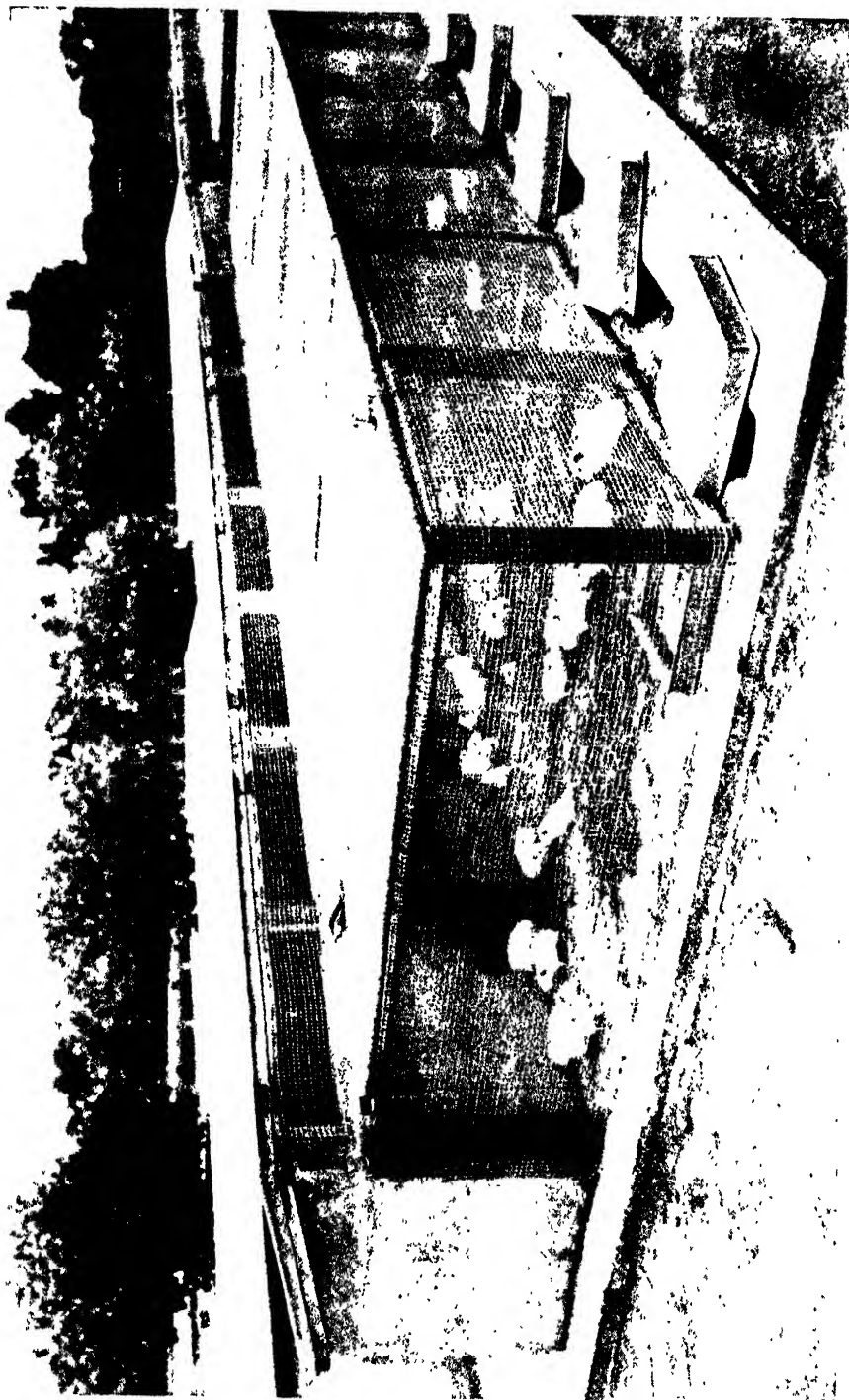
The chances of overcoming the inherent variability of the experimental material improve as the number of animals is increased. However, by means of inbreeding, it becomes possible to draw valid conclusions from smaller numbers of animals, as great uniformity is achieved. It was decided to utilise ten individuals for each weight-class. All observations recorded are, therefore, the means for ten rabbits within each group, with the exception of the groups from 100 gm. to 480 gm. live-weight, included later in the experiment. Here only five individuals were utilised for each group, as the data already collected seemed to justify this step.

In order to eliminate sex variation as a source of error, only buck rabbits were employed for this study. It is hoped it will be possible to compare muscle growth in does at some future date, to determine the effect of sex.

Environmental variations would have been minimised if all required experimental animals had been bred at the same time and reared together, until random selection of individuals for the different weight-groups. However, the facilities available rendered it impossible to breed and rear in this manner the large number of rabbits required. Consequently breeding was carried out the whole year round. Individuals were allocated at random whenever necessary. Each experimental group therefore comprised animals born at different seasons, and during different years for different groups.

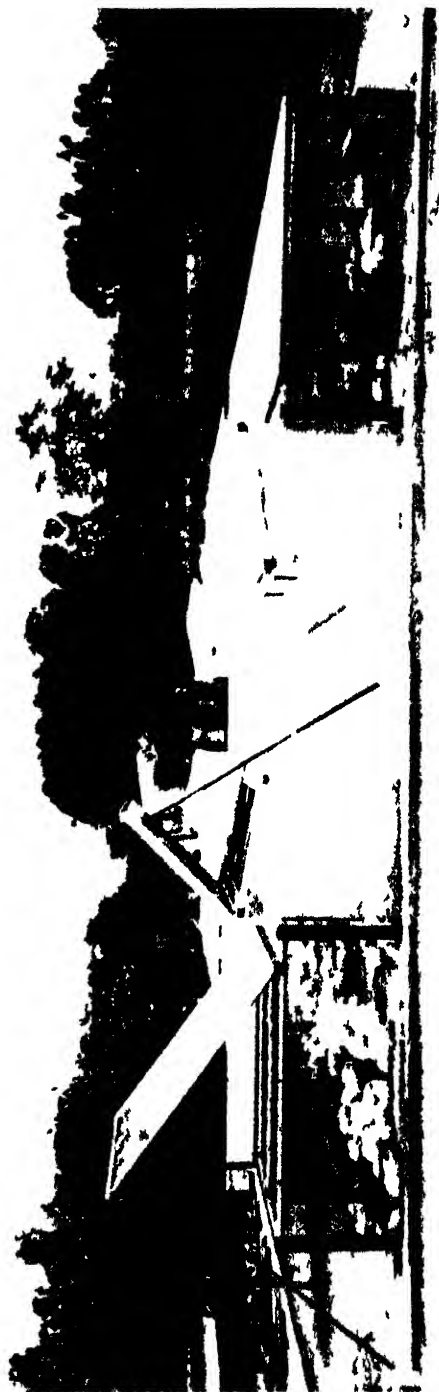
It was estimated it would not be possible to study more than two muscles by the methods proposed. The problem was which individual muscles should be selected for investigation. Both muscles should be capable of easy separation to permit of accurate measurement, yet, if possible, the muscles should have well-defined individual action. *M. Psoas major* was selected as a late developing muscle from the region of the back and loin. For contrast, *M. Gastrocnemius medialis* in the leg was chosen as the second muscle. Both muscles are easily identified and removed, moreover they represent different types of muscle.

M. Gastrocnemius medialis is a reddish muscle in which the muscle substance lies between two fascial layers; "one, an aponeurosis attached at its upper end to the femur and fading into muscle substance at its lower end; the other, an aponeurosis running into the *Tendo Achilles* below and



photograph taken in 1939, showing a general view of the small animal section. Similar accommodation is provided for rabbits.

PLATE II



Roof of hut and wire mesh cover of run in the raised position

fading into the muscle substance above." (Denny-Brown, 1929). Hundreds of fasciculi pass from the aponeurosis of origin (which is superficial), obliquely downwards and forwards to the aponeurosis of insertion. Hence fasciculus length is much shorter than muscle length in this case.

On the other hand, the pale Psoas major muscle is much larger than M. Gastrocnemius medialis. It is characterised by the largely parallel arrangement of its fibres along the long axis of the muscle, from origin to insertion. Origin is by means of a broad aponeurosis from the pleural surface of the third last rib, near the costal angle; also from the body of the last thoracic and all lumbar vertebrae, as well as from the transverse processes of the last-named. The muscle passes caudally along the long axis of the body, to unite with the terminal portion of M. Iliacus to form a common stout tendon inserted on the trochanter minor of the femur (Gerhardt, 1909).

Both M. Psoas major and M. Gastrocnemius medialis were studied as minutely as possible, in terms of the morphology of each functional unit. Firstly, the individual muscle was studied as a whole, in terms of weight, length, width, and depth; secondly, the individual bundle in terms of length and thickness; and lastly, the individual fibre, also as regards length and thickness.

(d) PROCEDURE.

1. Collection of data.

Rabbits were killed as soon as possible after they had attained the required weight. As Saturday was the regular day for weighing rabbits, it usually happened that the animals were killed on Monday morning. They were removed from the hutches before feeding, with as little disturbance as possible, to the laboratory a short distance away.

In order to minimise effects due to struggling, it was customary to anaesthetise the rabbit before bleeding it. Nembutal solution, utilised for this purpose, was injected intravenously at the rate of 0.2 c.c. per pound body weight. In addition, ether was lightly administered by means of a mask to deepen the degree of anaesthesia attained.

When relaxation was complete the carotid arteries and jugular veins were severed and the rabbit was suspended by the tail for fifteen to thirty minutes for drainage of the blood. At this stage the position was inverted, and the rabbit was suspended from both fore-limbs for approximately five hours. This measure was undertaken to ensure the hind legs and body were in a position as nearly reproducible as possible, during the period of rigor mortis allowed.

Selection of the period of rigor for five hours was arbitrary. It was designed to ensure loss of muscle irritability before handling, as well as to permit dissection and weighing of the muscles during the routine working hours. Data presented by Bate-Smith (1939) indicate it would be desirable to allow a longer period of rigor mortis. His work on the Psoas muscles from rabbits indicates that stiffening occurs up to eight hours after death, inclusive of an initial "normal" stationary period of two hours. Unfortunately it was not possible to utilise an eight-hour period. Leaving the animal overnight at room temperature did not offer a solution, as the degree of autolysis attained made it difficult to perform the required measurements on isolated muscle bundles. Experiments were also undertaken to achieve a reproducible standardised condition of the muscles, by perfusing the

anaesthetised animal with formalin solution. As living muscle reacts strongly to perfused fixative, this method too is not without disadvantage, and the method described was preferred.

After rigor mortis had progressed for five hours, the right Gastrocnemius medialis muscle was removed, weighed immediately, and immersed in neutralised ten per cent. Formalin solution in normal saline. Then followed in turn, the left Gastrocnemius medialis and right and left Psoas major muscles. Each muscle was removed from the fixative after 24 to 48 hours, rinsed in water, and the excess water mopped off the muscle. After measuring length of muscle to the nearest millimetre, the muscle was marked off into six equidistant portions demarcated as follows:—

Origin to "A", "A" to "B", "B" to "C", "C" to "D", "D" to "E", and "E" to insertion. In Figure 1, the procedure is explained in diagrammatic manner. Width of muscle was measured by means of a vernier caliper at points, A, B, C, D, E, after laying the muscle flat on top of the bench. Depth of muscle was measured by means of the same instrument, also at these five points, but the muscle was lifted for this measurement.

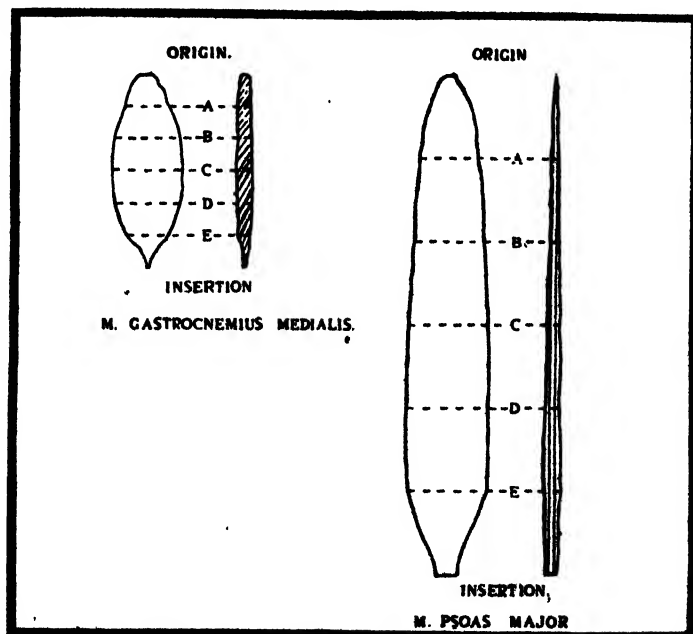


Fig. 1.—Sites at which width and depth of muscle were measured. The shading indicates the direction of muscle bundles on sectioning the muscle longitudinally.

Length of muscle bundle was always measured on the left muscle. In the case of M. Gastrocnemius, bundles were carefully teased out under water, at sites A to E, from a central longitudinal strip of muscle. Statistical methods were employed to ascertain suitability of sampling. These data, as well as the method of measurement, are presented later (page 355). The Psoas muscle could not be treated similarly because of the end-to-end arrangement of its component bundles. In this muscle it was only necessary to measure bundles along the length of the muscle.

Fibre diameter was determined on specimens removed from the right muscle, at sites A to E. A statistical evaluation of the suitability of method employed to measure fibre diameter is considered in detail at a later stage (page 360).

In addition, specimens were removed from the right muscle for histological purposes. These specimens were cut out immediately cranial to site C to ensure that each specimen was obtained at a relatively constant position and furthermore from the centre of the muscle where material is not scanty. "Grain", or texture of muscle was estimated from these pieces of muscle, by the method later described (pages 365 and 399). The specimens were washed in running water for several hours to remove excess Formalin solution. After infiltration with ten per cent. gelatin solution at 37° C. for roughly sixteen hours, the specimens were transferred to twenty per cent. gelatin for twenty-four hours, then imbedded in gelatin of like concentration. Setting of the gelatin was achieved by holding in a refrigerated chamber at roughly 1-5° C. for two to three hours. Excess gelatin was trimmed from the specimen, which was then transferred to cold ten per cent. neutral solution of Formol-saline to harden. Frozen sections were cut at 35 μ by means of a Jung microtome equipped with a carbon dioxide attachment.

Measurement of length of muscle bundle.

Preliminary work showed how laborious it would be, as a routine measure, to dissect out a large number of fasciculi in order to determine the mean length of the muscle bundles. It became necessary therefore, to determine the minimum number of measurements likely to afford a reasonably accurate estimate of bundle length.

In the case of the Gastrocnemius muscle, specimens were always obtained from a two millimetre wide central longitudinal strip of the muscle, in order to ensure a relatively constant site. After marking the superficial muscle surface at equidistant points to give three equal portions, the fasciculi in each third were carefully dissected out under water. Immediately after their removal, the bundles were straightened on the table top and calipers were used to measure their length to the nearest millimetre. Fifty random fasciculi were measured in each third of the muscle, and the data analysed.

Because of the end-to-end arrangement of bundles, the Psoas muscle could not be treated similarly. In this muscle a central strip was again used. The bundles pass right along the length of this strip, from which seventy bundles were removed at random for measurement.

These numbers are too large to be practical in experiments involving a number of animals. Hence the minimum number of measurements that can be used to give a mean value, that is representative of the muscle, was determined.

An analysis of the Gastrocnemius measurements is given in Tables 1 (A) and 1 (B), where the selection of fifty bundles at each site within the muscle is compared with selections of forty, thirty and twenty measurements. In addition, five differing selections each consisting of ten bundles are shown with their means and other statistical constants, as well as the results of the tests for significance. The Psoas measurements are presented in Tables 2 (A) and 2 (B), where seventy measurements are compared with selections of sixty, fifty, forty, thirty, and twenty, in addition to seven differing selections each consisting of ten measurements.

TABLE 1A.
Analysis of measurements of length of muscle bundles. Gastrocnemius muscle. No. 1.

Length of Bundle (mm).	Number of Bundles in Each Selection.									
	50.	40.	30.	20.	20.	10.	10.	10.	10.	10.
PROXIMAL THIRD.										
10.....	2	1	1	1	1	—	1	—	—	1
11.....	37	29	21	16	14	8	8	6	6	7
12.....	11	10	8	3	5	2	1	4	4	2
Mean (mm).....	11.2	11.2	11.2	11.1	11.2	11.2	11.0	11.2	11.4	11.1
Standard error.....	± .0681	± .0759	± .0920	± .1000	± .1170	± .1332	± .1491	± .1334	± .1633	± .1795
Standard deviation.....	.4818	.4798	.5041	.4472	.5232	.4217	.4714	.4217	.5164	.5676
Coefficient of variability.....	4.3%	4.3%	4.5%	4.0%	4.7%	3.8%	4.3%	3.8%	4.5%	5.1%
Significant differences.....	—	—	—	—	—	—	—	—	—	—
MIDDLE THIRD.										
10.....	1	1	1	1	—	—	—	—	1	—
11.....	24	19	16	10	9	3	5	6	4	6
12.....	25	20	13	9	11	7	5	4	5	4
Mean (mm).....	11.5	11.5	11.4	11.4	11.5	11.7	11.5	11.4	11.4	11.4
Standard error.....	± .0769	± .0876	± .0891	± .1338	± .1141	± .1527	± .1667	± .1663	± .2211	± .1633
Standard deviation.....	.5436	.5543	.5632	.5982	.5104	.4830	.5271	.5164	.6992	.5194
Coefficient of variability.....	4.7%	4.8%	4.9%	5.3%	4.4%	4.1%	4.6%	4.6%	6.1%	4.5%
Significant differences.....	—	—	—	—	—	—	—	—	—	—
DISTAL THIRD.										
11.....	1	1	1	—	1	—	—	—	—	1
12.....	27	22	18	13	9	7	4	7	5	4
13.....	19	15	8	5	9	3	5	2	4	5
14.....	3	2	3	2	1	—	1	1	1	—
Mean (mm).....	12.5	12.4	12.4	12.4	12.5	12.3	12.7	12.4	12.6	12.4
Standard error.....	± .0914	± .1010	± .1151	± .1535	± .1539	± .1527	± .2134	± .2211	± .2211	± .2211
Standard deviation.....	.6465	.6385	.7280	.6963	.6982	.4830	.6749	.6992	.6992	.6992
Coefficient of variability.....	5.2%	5.2%	5.9%	5.5%	5.5%	3.9%	5.3%	5.6%	5.6%	5.6%
Significant differences.....	—	—	—	—	—	—	—	—	—	—

TABLE 1B.
Analysis of measurements of length of muscle bundle. Gastrocnemius muscle. No. 2.

Length of Bundle (mm).	Number of Bundles in Each Selection.									
	50.	40.	30.	20.	20.	10.	10.	10.	10.	10.
PROXIMAL THIRD.										
11.....	11	10	8	4	4	—	1	1	4	5
12.....	28	22	17	10	12	7	9	6	4	2
13.....	11	8	5	6	4	3	—	3	2	3
Mean (mm).....	12.0	12.0	11.9	12.1	12.0	12.3	11.9	12.2	11.8	11.8
Standard error.....	± .0947	± .1071	± .1208	± .1606	± .1451	± .1527	± .1000	± .2000	± .2494	± .2008
Standard deviation.....	± .6698	± .6774	± .6817	± .7181	± .6488	± .4830	± .3162	± .6324	± .7888	± .9189
Coefficient of variability.....	5.6%	5.7%	5.6%	5.9%	5.4%	3.9%	2.7%	5.2%	6.7%	7.8%
Significant differences.....	—	—	—	—	—	—	—	—	—	—
MIDDLE THIRD.										
11.....	5	4	2	3	2	1	1	3	—	—
12.....	28	24	16	12	10	4	6	5	7	6
13.....	17	12	12	5	8	5	3	2	3	4
Mean (mm).....	12.2	12.2	12.3	12.1	12.3	12.4	12.2	11.9	12.3	12.4
Standard error.....	± .0885	± .0961	± .1109	± .1433	± .1469	± .2211	± .2000	± .2333	± .1627	± .1633
Standard deviation.....	± .6259	± .6076	± .6073	± .6407	± .6568	± .6991	± .6324	± .7379	± .4830	± .5163
Coefficient of variability.....	5.1%	5.0%	4.9%	5.3%	5.3%	5.6%	5.2%	6.2%	3.9%	4.2%
Significant differences.....	—	—	—	—	—	—	—	—	—	—
DISTAL THIRD.										
12.....	3	1	1	2	1	—	1	—	1	1
13.....	38	32	23	16	14	6	7	9	9	7
14.....	9	7	6	2	5	4	2	1	—	2
Mean (mm).....	13.1	13.2	13.2	13.0	13.2	13.4	13.1	13.1	12.9	13.1
Standard error.....	± .0679	± .0675	± .0844	± .1026	± .1170	± .1633	± .1795	± .1000	± .1000	± .1795
Standard deviation.....	± .4902	± .4266	± .4621	± .5588	± .5231	± .5163	± .5676	± .3162	± .3162	± .5676
Coefficient of variability.....	3.7%	3.2%	3.5%	3.5%	4.0%	3.9%	4.3%	2.4%	2.5%	4.3%
Significant differences.....	—	—	—	—	—	—	—	—	—	—

TABLE 2A.
Analysis of measurements of length of muscle bundle. Psoas muscle No. 1.

Length of Bundle. (mm).	Number of Bundles in Each Selection.													
	70.	60.	50.	40.	30.	30.	20.	20.	10.	10.	10.	10.	10.	10.
84.....	2	2	1	1	1	1	1	1	1	1	1	1	1	1
85.....	2	2	1	1	1	1	1	1	1	1	1	1	1	1
86.....	2	2	2	1	1	1	1	1	1	1	1	1	1	1
87.....	3	3	2	1	1	1	1	1	1	1	1	1	1	1
88.....	2	2	2	1	1	1	1	1	1	1	1	1	1	1
89.....	2	2	2	1	1	1	1	1	1	1	1	1	1	1
90.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1
91.....	2	2	2	2	1	1	1	1	1	1	1	1	1	1
92.....	3	3	1	1	1	1	1	1	1	1	1	1	1	1
93.....	3	3	2	2	2	2	1	1	1	1	1	1	1	1
94.....	5	5	2	2	2	2	2	2	2	2	2	2	2	2
95.....	8	8	5	6	3	4	4	4	3	3	3	3	3	3
96.....	5	5	4	3	3	3	3	3	3	3	3	3	3	3
97.....	11	9	6	7	4	5	1	1	1	1	1	1	1	1
98.....	7	5	6	6	3	3	2	2	1	1	1	1	1	1
99.....	2	2	1	3	1	1	1	1	2	2	2	2	2	2
100.....	6	6	5	3	2	2	1	1	1	1	1	1	1	1
101.....	4	4	3	1	2	2	1	1	1	1	1	1	1	1
102.....	2	2	2	2	2	2	1	1	1	1	1	1	1	1
Mean...	95.08 ± 5361	95.02 ± 6072	95.38 ± 6407	95.30 ± 6662	95.43 ± 7123	95.73 ± 8379	95.05 ± 8929	95.25 ± 9236	93.70 ± 10116	96.40 ± 10511	95.00 ± 10809	95.20 ± 116519	94.60 ± 115144	95.20 ± 116519
S. E....	4.4866	4.7030	4.5306	4.2135	3.9012	4.8632	3.9931	2.7886	3.1989	5.8538	2.9439	5.2238	4.7889	5.2238
S. D....	4.7%	5.0%	4.8%	4.4%	4.1%	5.1%	4.2%	2.9%	3.4%	6.1%	3.1%	5.5%	5.1%	5.5%
Sig. Dif.	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Abbreviations:—

S.E.....

S.D.....

C.V.....

Sig. Dif.....

Standard error.

Standard deviation.

Coefficient of variability.

Significant difference.

TABLE 2B.
Analysis of measurements of length of muscle bundle. Psoas muscle. No. 1.

Length of Bundle. (mm).	Number of Bundles in Each Selection.											
	70.	60.	50.	40.	30.	30.	20.	20.	20.	10.	10.	10.
90.....	2	2	—	2	1	—	—	—	—	—	1	1
91.....	10	10	9	3	5	—	2	—	—	—	2	1
92.....	7	6	6	4	2	4	1	3	1	1	—	2
93.....	11	8	10	9	4	5	6	1	1	2	2	—
94.....	13	12	8	7	6	5	1	5	2	3	2	4
95.....	7	6	5	5	2	4	4	1	1	1	1	—
96.....	5	4	3	1	2	3	3	1	1	1	2	1
97.....	7	5	3	5	3	3	1	4	1	—	1	—
98.....	3	3	3	1	3	3	2	1	—	—	1	—
99.....	3	3	3	3	1	—	—	—	—	2	—	—
100.....	1	1	1	—	1	—	—	—	—	—	—	—
101.....	—	—	—	—	—	—	—	—	—	—	—	—
102.....	1	1	1	—	—	1	—	—	—	—	—	—
Mean.....	94.23 ±.3121 2.8%	94.12 ±.3437 2.6622 2.8%	94.04 ±.3681 2.6027 2.8%	94.18 ±.3821 2.4166 2.6%	94.37 ±.4971 2.7226 2.9%	94.23 ±.4641 2.5419 2.7%	93.40 ±.5099 2.2804 2.4%	94.55 ±.5780 2.5850 2.7%	95.10 ±.6085 2.7125 2.9%	93.60 ±.6182 1.9550 2.1%	94.80 ±.8794 2.7808 2.9%	94.30 ±.7608 2.4060 2.6%
S. E.....	93.30 ±.6506	93.70 ±.6289 2.1%	93.80 ±.6289 2.1%	94.10 ±.6741 2.3%	95.70 ±.1.3337 4.4%	93.80 ±.6289 2.1%	94.30 ±.7608 2.6%	94.30 ±.7608 2.6%	94.30 ±.7608 2.6%	94.30 ±.7608 2.6%	94.30 ±.7608 2.6%	94.30 ±.7608 2.6%
S. D.....	2.0875	2.0875	2.0875	2.0875	2.0875	2.0875	2.0875	2.0875	2.0875	2.0875	2.0875	2.0875
C. V.....	2.2%	2.2%	2.2%	2.2%	2.2%	2.2%	2.2%	2.2%	2.2%	2.2%	2.2%	2.2%
Sig. diff.....	—	—	—	—	—	—	—	—	—	—	—	—

Abbreviations:—

S.E.....

S.D.....

C.V.....

Sig. Diff.....

Standard error.

Standard deviation.

Coefficient of variability.

Significant difference.

No significant differences are shown among the means obtained from these various selections of measurements.; Furthermore, for each selection of ten measurements, the probable range of the mean does not exceed three per cent., which falls within the five per cent. level regarded as a satisfactory biological standard (Table 3). It was concluded that a reasonably accurate measurement of length of muscle bundle is obtained by measuring ten bundles. Accordingly all calculations in the growth study were based on an average value obtained from ten bundles.

TABLE 3.

Probable range of mean bundle length. Analysis of variance calculated at the 5 per cent. level of probability for five (or seven) samples of ten measurements each, in two muscles.

Muscle.	Number of Measurements.	Mean Bundle Length (mm.).	Probable Range of Means of 10 Measurements.
<i>Gastrocnemius No. 1.—</i>			Per Cent.
Proximal third.....	50	11.18	2.30
Middle third.....	50	11.48	2.57
Distal third.....	50	12.48	2.80
<i>Gastrocnemius No. 2.—</i>			
Proximal third.....	50	12.00	2.94
Middle third.....	50	12.24	2.70
Distal third.....	50	13.12	1.90
<i>Psoas No. 1.....</i>	70	95.03	2.57
<i>Psoas No. 2.....</i>	70	94.28	1.46

In both *Gastrocnemius* muscles the shortest bundles are obtained from the proximal third of the muscle. The longest bundles are found in the distal third, with bundles from the mid-portion occupying an intermediate position.

In order to examine more closely this differential relationship in length of bundle of *M. Gastrocnemius*, measurements in the growth study were made at five points (A to E as previously described), not along thirds of muscle as for this preliminary work. In addition, a binocular head-band magnifier was used in conjunction with a vernier caliper for dissociating and measuring bundles. By this means experimental accuracy has been increased, and the labour eased enormously.

Measurements of fibre diameter.

Hammond's technique, of measuring the diameter across short lengths of teased out formalin-fixed fibres, appears to be suitable for the purposes of this experiment. However, as cross-sections of muscle can be utilised for the study of fasciculi, fat, connective tissue, in addition to fibre diameter, it was decided to compare the sectional method with Hammond's method.

Three aspects were considered in making this comparison; namely suitability of method for measuring fibre diameter, the number of measurements which affords a representative sample of the fibre population, and the variation of fibre diameter within a muscle.

At the time the observations were made, formalin-perfused muscle was employed for experimental purposes. The tissues used in the growth study were all fixed only after removal from the carcass, five hours post-mortem. Nevertheless, it is believed the value of these preliminary results is not impaired.

Immediately after formalin perfusion of the anaesthetised rabbit, the right *Gastromedialis* and *Psoas major* muscles were removed. Five slices were cut from each muscle at the equidistant points A to E. After fixation in neutralised ten per cent, formal saline solution, the slices were imbedded in gelatin according to the method of Zwemer (1933). Transverse sections were cut on a freezing microtome at 35μ and mounted in glycerin jelly without staining. Subsequently, the same gelatin-imbedded muscle slices were used for teasing out short lengths of isolated muscle fibres by Hammond's technique.

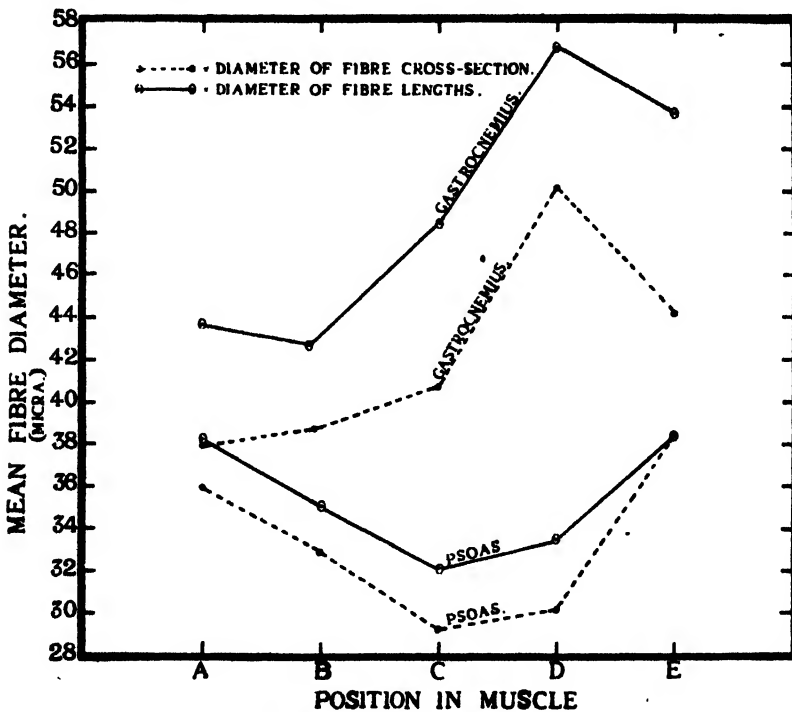


Fig. 2.—Fibre diameter.

Measurement of diameter was made by means of a Zeiss Lanameter at a magnification of 500 times. Both the vertical and horizontal dimensions of the transversely cut fibres were measured in the muscle sections. 250 fibres were measured in this way and the mean diameter determined. In the case of the teased preparations 250 measurements were also made, but only in terms of the diameter across loose fibres.

Each group of 250 measurements was randomised to give two selections of 125, two of 100, three of 75, five of 50, and ten of 25 measurements each. These data were analysed statistically at the five per cent. level of probability to assess the relative value of the two methods.

Discussion.

The diameter of fibres at points A to E, as well as the statistical constants, for both the Gastrocnemius and Psoas muscles are given in Appendix Tables A, B, C and D.

Fibre diameter along the length of the muscle is depicted in Figure 2. In *M. Gastrocnemius*, there is little difference in fibre diameter at points A and B. Thereafter, there is a progressive increase to C, and to D. Near the tendinous insertion of the muscle, the diameter decreases from D to E. A well-defined gradient exists within the muscle. The smallest fibres are found near the origin of the muscle, and the largest nearer to the insertion. In *M. Psoas*, however, the thinnest fibres are found about the middle of the muscle, and the thickest fibres at both ends of the muscle. Furthermore, the *Psoas* fibres are roughly only two-thirds as thick as those from *M. Gastrocnemius*. Thus the architecture is markedly different in the two muscles.

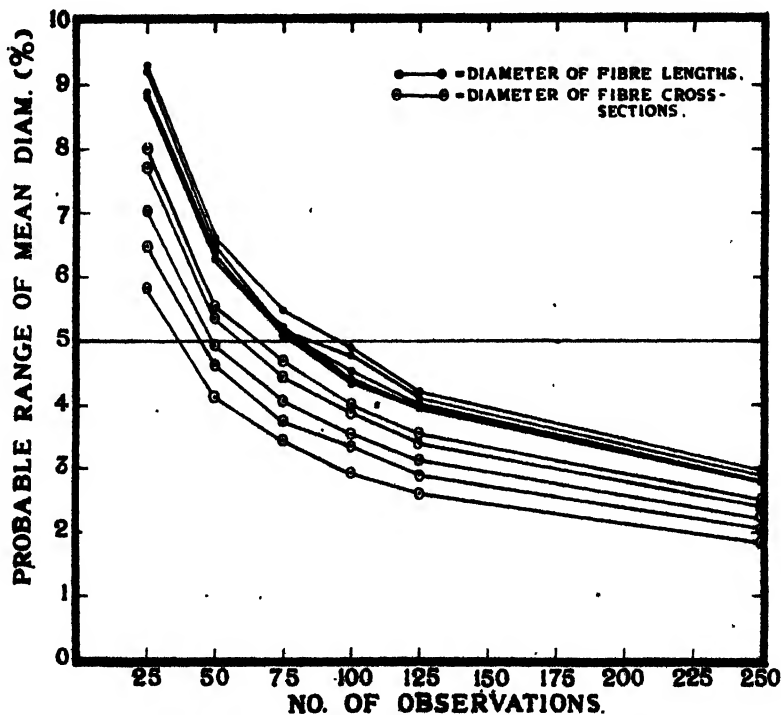


Fig. 3.—Percentage probable range of mean fibre diameter of *M. Gastrocnemius*.

Although the curves for fibre diameter obtained by Hammond's method are nearly parallel to those obtained from cross-sections, the diameter of the teased fibres is greater at all points. The reason is obscure. If the muscle fibres tend to be oval rather than circular, it is possible the teased out fibres settle on the longer axis, which is then always measured. Other factors may be the application of the coverslip to the slide, and the subsequent setting of the mounting medium. Both may tend to flatten the

teased fibre and produce an apparent thickening. This difference in diameter is an indication that care must be taken in mounting the coverslip, so as not to exercise undue pressure on the fibres comprising the specimen.

In Table 4 and Figure 3 are considered the relationship between the number of measurements and the percentage probable range of the mean diameter. As the number of observations making up a group is increased, the probable range of the mean diameter is decreased; markedly from groups of 25 to 50, less pronounced from 50 to 75, and in more gradual manner for the larger groups of 100, 125 and 250 observations.

TABLE 4.

Probable range of mean fibre diameter. Analysis of variance calculated at the 5 per cent. level of probability for selections of 250, 125, 100, 75, 50 and 25 measurements each, Rabbit No. 16.

Muscle.	Number of Measurements.					
	250.	125.	100.	75.	50.	5.
	%	%	%	%	%	%
Right Gastroc. (Cross-section).						
Site A.....	2.21	3.13	3.55	4.06	4.93	7.03
Site B.....	1.85	2.61	2.92	3.43	4.13	5.83
Site C.....	2.40	3.38	3.88	4.44	5.35	7.71
Site D.....	2.50	3.64	4.00	4.68	5.54	8.01
Site E.....	2.06	2.90	3.34	3.73	4.64	6.48
MEAN.....	2.20	3.11	3.54	4.07	4.92	7.01
Right Gastroc. (Hammond's Method).—						
Site A.....	2.97	4.17	4.91	5.48	6.62	9.30
Site B.....	2.80	3.95	4.36	5.11	6.27	8.89
Site C.....	2.79	3.93	4.40	5.22	6.26	8.81
Site D.....	2.89	4.10	4.78	5.17	6.48	9.23
Site E.....	2.83	4.00	4.52	5.13	6.37	8.81
MEAN.....	2.85	4.03	4.59	5.22	6.40	9.01
Right Psoas (Cross-section).—						
Site A.....	2.18	3.09	3.37	4.05	4.85	6.96
Site B.....	2.10	2.96	3.25	3.84	4.68	6.56
Site C.....	2.48	3.50	3.90	4.60	5.48	7.85
Site D.....	2.62	3.71	4.16	4.83	5.89	8.38
Site E.....	1.65	2.33	2.69	3.01	3.68	5.18
MEAN.....	2.21	3.12	3.47	4.07	4.92	6.99
Right Psoas (Hammond's Method).—						
Site A.....	2.51	3.54	3.91	4.56	5.63	7.95
Site B.....	2.83	4.01	4.25	5.22	6.37	8.99
Site C.....	3.41	4.82	4.96	6.13	7.48	10.62
Site D.....	2.72	3.86	4.41	5.06	6.09	8.67
Site E.....	2.88	3.37	3.79	4.51	5.33	7.46
MEAN.....	2.77	3.92	4.26	5.10	6.18	8.74

In the cross-sections, the range exceeds five per cent. for the 25 and 50 groups, but is consistently below the five per cent. level for the groups of 75 measurements, and shows a further slight progressive decrease for the 100, 125 and 250 groups. In the teased preparations, the probable range falls below five per cent only for 100 measurements, as compared with 75 for the cross-sections. However, this reduction in measuring cross-sections is not real, as 150 readings (75 horizontal plus 75 vertical) were taken to obtain the mean cross-sectional diameter for 75 fibres.

In addition, whereas the probable range for the cross-sections varies widely from specimen to specimen, it is relatively constant for the teased fibres. Figure 3 clearly depicts this variability. Such variability may be explained by the experimental difficulty of cutting a truly transverse section. Although care is exercised in aligning the fibres, a reproducible degree of cross-sectioning cannot be obtained in different specimens. This technical difficulty falls away when fibres are teased out according to Hammond's method.

An assessment of the number of fibres which will afford a representative sample largely depends on the degree of accuracy required. For the purpose of this experiment, a measurement of diameter was considered sufficiently accurate, for which the probable range of the mean diameter does not exceed five per cent. This standard of accuracy is achieved by measuring the diameter of at least 100 fibres teased from the muscle specimen. Such a sample is representative of the fibre population and affords reasonably accurate results.

In another investigation of fibre diameter, 500 measurements were made of teased fibres from a number of different specimens. The analysis of these results is presented in Table 5. As the results are essentially similar to those described above, these data are not considered in detail. It suffices to state, these additional calculations confirm the finding that the mean diameter of 100 teased fibres has a probable range of not more than five per cent.

TABLE 5.

Probable range of mean fibre diameter. Analysis of variance calculated at the 5 per cent. level of probability for selections of 500, 250, 125, 100 and 75 measurements.

Muscle.	Number of Teased Fibres Measured.				
	500.	250.	125.	100.	75.
Gastrocnemius—	%	%	%	%	%
Sample 1.....	2.08	2.99	4.12	4.58	5.31
Sample 2.....	2.03	2.84	3.97	4.44	5.12
Sample 3.....	2.21	3.15	4.56	5.00	5.66
Sample 4.....	2.03	3.03	4.28	4.54	5.30
Sample 5.....	2.06	2.83	3.96	4.72	5.33
Sample 6.....	1.98	2.78	3.73	4.59	4.87
MEAN.....	2.07	2.94	4.10	4.65	5.27

As there is no doubt that Hammond's method of measuring teased fibres is preferable to a sectional method, his technique was applied throughout the growth study. Mean diameter was always calculated from measurement of 100 fibres. In making the selection at random, the method used for wool fibres by Bosman and van Wyk (1939) was followed.

Measurement of texture of muscle.

In order to obtain a measure of muscle texture it is necessary to calculate how many fibres constitute a muscle bundle, as well as the average thickness of these individual fibres.

The fibres were counted in a large number of bundles in each of several muscles, utilising the transverse sections prepared for that purpose as described. These data were subjected to statistical analysis by means of artificial stratified sampling. It was concluded that the error made by using five to ten bundles in a muscle will, on the whole, be the same as that obtained by using twenty bundles. To remain on the safe side, it was decided to select twenty random bundles from each muscle, to afford an average count of the number of fibres constituting the muscle bundle. Hence all calculations in this study are based on an average value obtained from twenty bundles.

(2) Treatment of data.

In order to afford a basis of comparison it is necessary that the experimental data be arranged in groups. Either age or live-weight of the animals affords a method of classification. However, there are indications, both on practical and on theoretical grounds, that age is less satisfactory than weight as a standard of comparison.

In Figure 4 a diagram is given representing both age and live-weight of the animals utilised for this experiment. A glance indicates the variability of the age of animals comprising any one group. Between the groups, there is no clear demarcation evident on an age basis, except for the two final groups. By comparison, greater uniformity of live-weight of animals exists within each group. Moreover, there is a clear spacing of all the groups except for the mature animals, as is to be expected.

In order to arrive at simpler indications of the growth processes there appears to be little doubt that the data should be grouped according to weight of the animal. It is to be emphasised, however, that this will result in the two final groups being plotted very closely adjacent in the curves illustrating growth changes. This is true in terms of body weight (3017–3072 Gm.). However, the last group was derived by holding these animals for a period of six months after they had attained a live-weight of 3,000 Gm., so that these rabbits are considerably older than the preceding group.

The data were grouped in twelve groups according to the average live-weight of the rabbits composing each group. Fisher's (1941) "Analysis of Variance" method was used to test the means for each of these groups, the Z test being employed to determine the existence of significant differences,

while the significantly differing groups were picked out by means of the *t* test. In comparing relative values within the muscle, the means for the various sites (A, B, C, D, E,) were calculated in each group, and these were tested in similar manner.

As standards of significance were regarded the values of *Z* and *t*, when $P=0.05$ (i.e. 5 per cent. probability), and when $P=0.01$ (i.e. 1 per cent. probability). *X* indicates a positive result at the former level (which already indicates definite significance), while a similar result at the higher level of significance is indicated by *XX*.

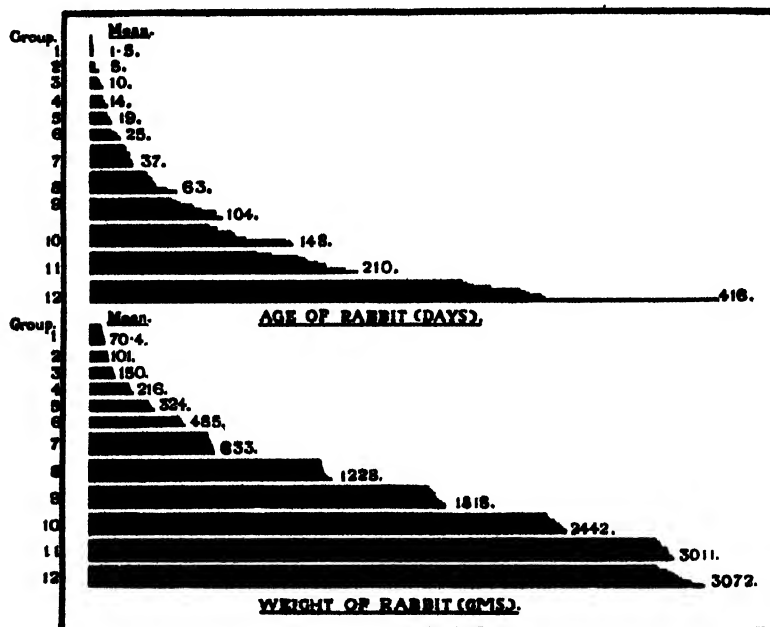


Fig. 4.—Age and live-weight of the experimental animals. Graph compiled from data in Appendix, Table E.

The complete data are shown in a series of tables in the Appendix. For the graphs, smoothed values of the observed mean values were calculated by means of the method of least squares. Smooth curves constructed in this manner are employed in the figures, but the distribution of the actual observed means about the curves is also plotted. Only the observed means for the sites and groups are employed in the tables. In these tables are also indicated the number of each group, the class (or description), and the number of rabbits in each group. Then the mean for the site or group is stated, and the results of the tests for significance. For the intra-muscular analysis (i.e. sites), the results of these tests are given in two columns, the first indicating the results when each site is tested against the one immediately preceding, while the second column shows the results of testing each site against the first site (i.e. "A"). For the inter-muscular analysis, (i.e. groups), the second column is omitted as it has little application.

Huxley's law of simple allometry has been used throughout to express the relation of the measurement of the part to the measurement of the whole, in this case the body-weight of the rabbit. This is written:—

$$y = bx^a \dots \dots \dots (1)$$

where y = measurement of the part,
 x = measurement of the whole,
 b = a constant,
 a = the equilibrium constant.

$$\text{or, log. } y = \text{log. } b + a \text{ log. } x \dots \dots \dots (2)$$

First, the individual observations were translated to logarithmic form in order to test the linear relationship in logs. For purposes of computation expression (2) was formulated as $\hat{Y} = B + AX \dots \dots \dots (3)$

where \hat{Y} = best estimate of $Y + \text{log. } y$,
 $X = \text{log. } x$,
 $B = \text{log. } b$,
 A = best estimate of a .

The plotting of $\text{log. } y$ against $\text{log. } x$ did not result in a straight-line distribution for certain measurements. Hence a logarithmic parabola was fitted to all measurements as a first empirical extension of the allometric formula, thus—

$$\hat{Y} = B + AX + CX^2 \dots \dots \dots (4)$$

as equivalent of

$$\hat{y} = bx^a 10^{c \log.^2 x} \dots \dots \dots (5)$$

$$\text{or, } \hat{y} = bx^{a + c \log. x} \dots \dots \dots (6)$$

By means of the method of least squares the equation was fitted to the data. From the anti-logarithms the corresponding expected values of y were determined for the series of values of body weight x . In this way smooth muscle—body-weight curves were constructed for the data.

As the same set of body-weights was used throughout the experiment Fisher's (1941) technique was used to avoid solving the simultaneous equations afresh on each occasion. By introducing Waugh's (1935) method of solving the equations further simplification was possible. After obtaining the numerical values of the constants B , A and C , the last-mentioned was tested for significance. If insignificant, the last term in (4) was omitted and the necessary corrections applied to A and B .

The instantaneous rate of increase relative to body-weight was also determined by mathematical differentiation of the allometric formula, or expression (4), the formulae becoming respectively

$$\frac{dy}{dx} = A \frac{y}{x} \dots \dots \dots (7)$$

$$\text{and } \frac{dy}{dx} = (A + 2CX) \frac{\hat{y}}{x} \dots \dots \dots (8)$$

CHAPTER IV.—OBSERVATIONS.

(a) GROWTH AND DEVELOPMENT OF THE RABBIT.

(Literature: Pages 335 to 337.)

Animal growth and development fall outside the scope of this investigation, except with reference to the basal study of muscle morphology. Hence, animal growth is only considered insofar as it provides a background of the rabbits used for the observations concerning muscle growth and development.

Live-weight with increasing age, and weekly rate of growth as measured in grams per week increase, are shown in Figure 5. These have been calculated from the average columns of Table 6.

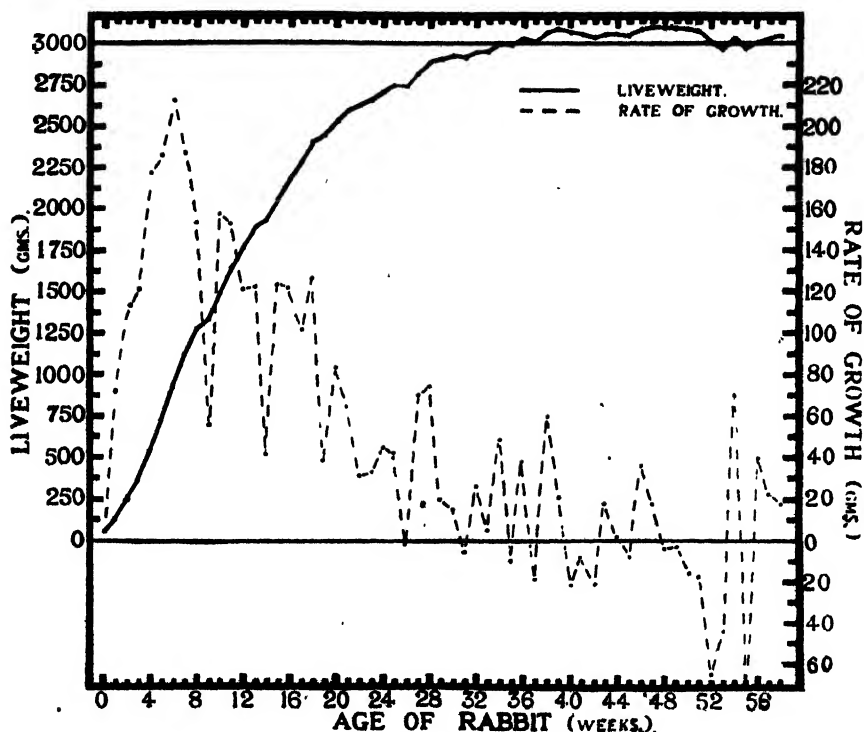


Fig. 5.—Live-weight growth and rate of growth.

Live-weight growth follows the conventional pattern determined for various species. At thirty-four weeks of age the live-weight first reaches a level of 3,000 gm. followed by fluctuation up to a maximum of 3,104 gm. at forty-seven weeks. Thereafter, live-weight fluctuates until fifty-eight weeks of age, when the weight recorded is 3,038 gm. As 3,000 gm. was estimated to be the approximate upper limit of live-weight, it is of interest to see that this agrees with the data compiled from the experimental animals (mean live-weight from thirty-four to fifty-eight weeks is 3,041 gm.). Attention must be directed to the decreasing number of rabbits towards the end of the growth curve. There are less than ten individuals from thirty-seven weeks. This reduction in number of animals should be taken into consideration in evaluating the data, especially near the end of the growth period.

TABLE 6.
Average weight of rabbits.

Age. (Weeks).	No. of Rabbits.	Weight (Gm.).	Weekly gain. (Gm.).	Age. (Weeks).	No. of Rabbits.	Weight (Gm.).	Weekly gain. (Gm.).
Birth.....	50	61	—	30.....	15	2925	15
1.....	63	133	72	31.....	15	2919	-6
2.....	54	241	108	32.....	13	2945	26
3.....	41	362	121	33.....	11	2950	5
4.....	37	540	178	34.....	10	2999	49
5.....	33	726	186	35.....	12	2989	-10
6.....	33	939	213	36.....	10	3027	38
7.....	25	1126	187	37.....	9	3008	-19
8.....	19	1280	154	38.....	9	3068	60
9.....	24	1336	56	39.....	9	3089	21
10.....	23	1494	158	40.....	9	3067	-22
11.....	30	1647	153	41.....	8	3059	-8
12.....	34	1768	121	42.....	9	3038	-21
13.....	31	1891	123	43.....	10	3066	18
14.....	33	1933	42	44.....	8	3058	2
15.....	31	2057	124	45.....	10	3050	-8
16.....	27	2179	122	46.....	9	3086	36
17.....	28	2281	102	47.....	10	3104	18
18.....	22	2408	127	48.....	10	3100	-4
19.....	26	2447	39	49.....	9	3097	-3
20.....	23	2531	84	50.....	10	3081	-16
21.....	22	2596	65	51.....	9	3064	-17
22.....	21	2627	31	52.....	8	2999	-65
23.....	19	2660	33	53.....	6	2955	-44
24.....	22	2705	45	54.....	7	3025	70
25.....	19	2747	42	55.....	6	2957	-68
26.....	21	2745	-2	56.....	6	2997	40
27.....	18	2815	70	57.....	5	3020	23
28.....	16	2890	75	58.....	6	3038	18
29.....	16	2910	20				

Rate of growth increases up to about the sixth week of life, rising from a live-weight gain of 72 gm. in the first week to 213 gm. in the sixth week. After this the growth rate decreases. A negative quantity (-2 gm.) is recorded at twenty-six weeks. Thereafter, until the fifty-eighth week, rate of growth fluctuates with roughly alternating periods of slight gain or loss of weight.

(b) GROWTH AND DEVELOPMENT OF MUSCLE.

1. *Weight.*

(Literature: pages 337-338.)

The mean muscle weights for each of the twelve groups are presented both in tabular and in graphic form (Table 7 and Figure 6).

At birth the Psoas muscle is twice as heavy as M. Gastrocnemius. This difference becomes increasingly more marked as the animal increases in weight, so that in the mature animal M. Psoas is approximately four times heavier than M. Gastrocnemius. Significance is not shown for the small cumulative increases in the earlier groups. All increments are, however, significant in M. Gastrocnemius from 320 gm. live-weight onwards, except for the mature group, and in the Psoas muscle from 600 gm. live-weight until maturity.

TABLE 7.
Weight of muscle.

GROUPS OF RABBITS.		No. of Rabbits.	Mean Weight M. Gastroc.	Sig. Test (W. Prec. Group).	Mean Weight M. Psoas.	Sig. Test (W. Prec. Group).
No.	Class.					
1.....	Birth.....	10	Gram. 0.048	—	Gram. 0.109	—
2.....	100	5	0.056	N.S.	0.156	N.S.
3.....	150	5	0.117	N.S.	0.268	N.S.
4.....	220	5	0.226	N.S.	0.431	N.S.
5.....	320	5	0.424	N.S.	0.870	N.S.
6.....	480	5	0.747	X	1.564	N.S.
7.....	600	10	1.00	X	1.95	N.S.
8.....	1200	10	1.90	XX	4.60	XX
9.....	1800	10	2.95	XX	8.60	XX
10.....	2400	10	3.71	XX	11.55	XX
11.....	3000	10	4.64	XX	14.79	XX
12.....	Mature....	10	4.72	N.S.	16.13	XX

Doubt may be expressed as to why the weight increments do not show significance in the early stages. Although a well-marked live-weight difference exists in the animals from which these muscles were derived, it is known that the major portion of the increase in weight of the young animals may be attributed to the early developing tissues and systems, e.g., skin, bone, head, feet. By comparison with these tissues and organs, muscle makes a greater proportion of its growth later in life.

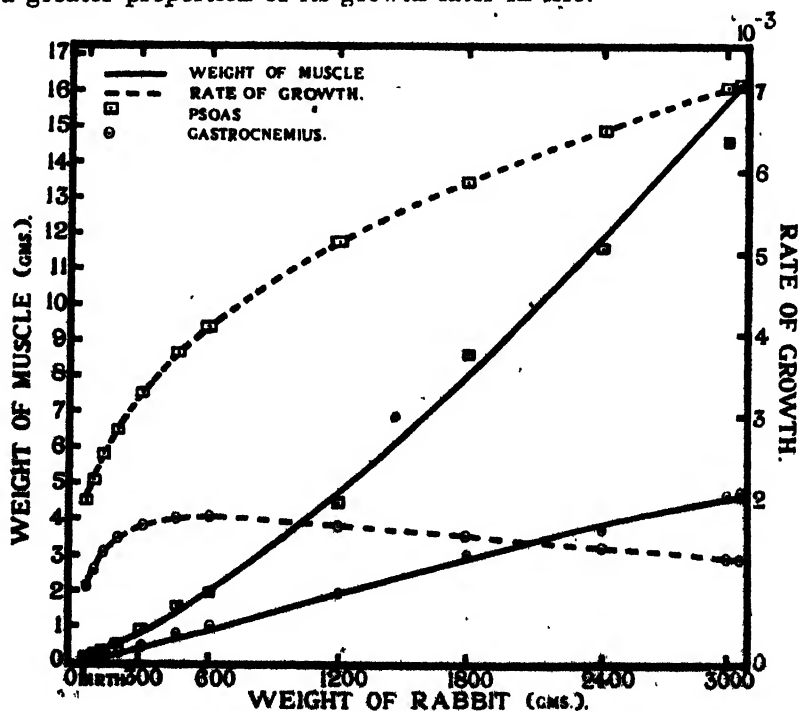


Fig. 6.—Weight of muscle.

Having demonstrated this increase in mass, it is of interest to consider the rate at which the additional muscle substance is accumulated (Table 8 and Figure 6).

It is seen that the rate of growth* of *M. Gastrocnemius* increases sharply until 220 gm. live-weight. After a subsequent more gradual rise until 600 gm., the growth rate then subsides slowly until 3,000 gm. live-weight, and shows a negligible decrease for the last group.

In the *Psoas* muscle there is a steady increase in the rate of growth, making for great dissimilarity between the two curves. At birth, the rate of growth of *M. Psoas* is approximately twice as great as that for the *Gastrocnemius* muscle. From this point the rate shows a steep upward trend until 600 gm. live-weight. Unlike *M. Gastrocnemius*, which subsides at this stage, the *Psoas* growth rate continues to increase until 3,000 gm. live-weight, although in a more gradual manner than in the initial stages. Even in the last group there is a slight final increase. Thus, in the mature animal the rate of growth of the *Psoas* muscle is more than five times that of *M. Gastrocnemius*.

TABLE 8.
Rate of growth of muscle weight.

Group.	M. Gastrocnemius.	M. Psoas.	Group.	M. Gastrocnemius.	M. Psoas.
Birth.....	0.000,920	0.001,942	Gram.		
100	0.001,128	0.002,214	600	0.001,782	0.004,116
150	0.001,338	0.002,529	1200	0.001,687	0.005,149
220	0.001,515	0.002,861	1800	0.001,540	0.005,881
320	0.001,669	0.003,282	2400	0.001,396	0.006,499
480	0.001,762	0.003,761	3000	0.001,282	0.006,975
			Mature.....	0.001,270	0.007,020

Discussion.

It is evident that the mode of growth is different in these two muscles. Whereas *M. Gastrocnemius* is an early developing muscle making a great proportion of its growth early in life, the *Psoas* muscle achieves maturity late in the lifetime of the animal. Until this growth has been analysed in terms of the muscle dimensions (length, width, depth), it cannot be decided what share can be attributed to each of these characters.

2. Length.

(Literature: pages 338-339.)

Length is considered in Table 9 and Figure 7.

M. Psoas is appreciably longer than *M. Gastrocnemius* at birth. Throughout the growth of the animal this difference becomes accentuated, so that *M. Psoas* is roughly ten centimetres longer than the *Gastrocnemius* muscle at maturity. Nevertheless, the relative proportions are more or less the same throughout the life of the animal.

* Rate of growth indicates the instantaneous rate of increase as determined by the formula on page 367.

TABLE 9.
Length of muscle.

GROUPS OF RABBITS.		No. of Rabbits.	Mean Length M. Gastroc.	Sig. Test (W. Prec. Group).	Mean Length M. Psoas.	Sig. Test. (W. Prec. Group).
No.	Class.					
1.....	Birth.....	10	Cm. 0.99	—	Cm. 2.72	—
2.....	Gram. 100	5	1.08	N.S.	3.58	N.S.
3.....	150	5	1.50	XX	4.30	N.S.
4.....	220	5	2.04	XX	5.16	N.S.
5.....	320	5	2.50	XX	6.62	XX
6.....	480	5	3.00	XX	8.04	XX
7.....	600	10	3.27	X	8.85	N.S.
8.....	1200	10	4.12	XX	10.17	XX
9.....	1800	10	5.00	XX	12.44	XX
10.....	2400	10	5.48	XX	14.50	XX
11.....	3000	10	5.93	XX	15.82	XX
12.....	Mature....	10	6.15	X	15.96	N.S.

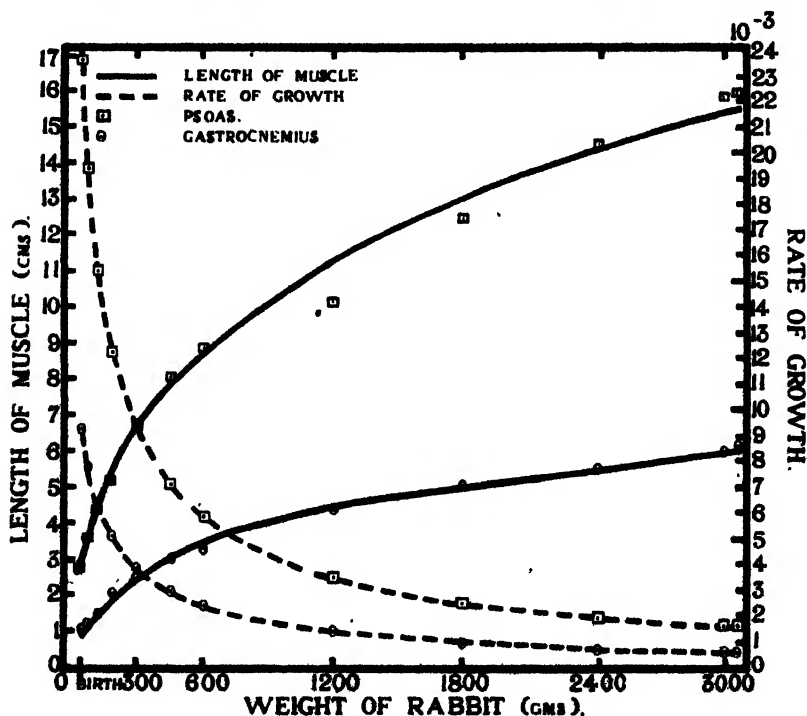


Fig. 7.—Length of muscle.

During each stage of growth from 100 gm. live-weight onwards, significantly differing amounts are added to the length of M. Gastrocnemius. Increments accruing throughout growth of M. Psoas do not show the same regularity of significance. In the earlier stages up to 220 gm. live-weight at 600 gm., and in the mature rabbit the variations show a negative result. This is probably partly explained by the inherent difficulty of measuring the

length of a severed muscle, aggravated further by the frailty of the Psoas origin. Here the boundary between attachment and muscle tissue proper is indefinite. Consequently, damage to the slight muscle in this locality with resultant measurement of an apparently shorter unit cannot be excluded. On the other hand, a firm bone to tendon attachment of M. Gastrocnemius at its origin, together with its clearly demarcated terminal passage from muscle to tendon, facilitate separation of this muscle as a unit.

Details concerning the rate of length growth are presented in Table 10 and Figure 7. Rate of growth of M. Psoas is larger than that for M. Gastrocnemius throughout the period studied. In both muscles the rate at birth is greater than for any subsequent group. At this stage the Psoas growth rate is nearly three times as large as that for M. Gastrocnemius. Subsequent to birth, the Psoas curve falls almost vertically until 480 gm. live-weight, after which it falls off more gradually. Although the Gastrocnemius curve shows the same general trend, the flattening out is more gradual throughout. Both curves conclude on a nearly parallel course with only a slight decrease in rate of growth in the last few groups.

TABLE 10.
Rate of growth of muscle length.

Group.	M. Gastrocnemius.	M. Psoas.	Group.	M. Gastrocnemius.	M. Psoas.
Birth.....	0.009,219	0.023,587	Gram.		
Gram.			600	0.002,382	0.005,812
100	0.007,755	0.019,366	1200	0.001,363	0.003,458
150	0.006,273	0.015,369	1800	0.000,941	0.002,484
220	0.005,055	0.012,241	2400	0.000,697	0.001,913
320	0.003,887	0.009,371	3000	0.000,557	0.001,579
480	0.002,921	0.007,072	Mature.....	0.000,544	0.001 550

Discussion.

It is well known that the vertebral column in the ox, sheep, and pig, has a larger rate of growth than the bones of the limb, particularly for the lumbar and sacral regions in which M. Psoas is situated. Hence, it is to be expected that the Psoas muscle will lengthen to a relatively greater degree than M. Gastrocnemius. If length is considered as a percentage of the length of each muscle at birth, it is seen that the relative proportions are more or less the same throughout the period studied. The respective figures are as follows:—

	Relative Length of Muscle with Growth.					
	Birth.	100 gm.	150 gm.	220 gm.	320 gm.	480 gm.
M. Gastrocnemius.....	100	109	152	206	253	303
M. Psoas.....	100	132	158	190	243	296
	600 gm.	1200 gm.	1800 gm.	2400 gm.	3000 gm.	Mature.
M. Gastrocnemius.....	330	410	500	550	590	620
M. Psoas.....	324	375	460	533	581	588

Perhaps a species difference is responsible for the discrepancy, on account of the specialised mode of progression in the rabbit. This possibility is enhanced by the fact that Appleton has measurements in the domestic rabbit, "which show that the growth in length of the tibia after birth is for a time considerably more rapid than that of the femur" (Hammond and Appleton, 1932, p. 378.) This is in contrast with the other domestic animals in which the reverse is true. As length of the Gastrocnemius muscle may be considered equivalent to length of tibia, which increases to a relatively greater extent than in other animals, the explanation advanced of a species difference is likely to be correct.

Another possibility is that, as a consequence of formalin fixation, the Psoas muscle incurs a greater degree of shrinkage in length than M. Gastrocnemius. Such shrinkage would tend to obscure the experimental results. By virtue of the longitudinal arrangement of the Psoas fibres as compared with a pinnate structure in the Gastrocnemius muscle, it is possible that fixation may induce a differential effect on length of muscle.

3. *Width.*

(Literature: Page 339.)

Width of muscle is considered as a general measurement. For this purpose the width has been calculated as a mean value of measurements at the five sites measured, and compared throughout the period of lifetime studied in the rabbit (Table 11).

TABLE 11.
Width of muscle.

GROUPS OF RABBITS.		No. of Rabbits.	Mean Width M. Gastroc.	Sig. Test (W. Prec. Group).	Mean Width M. Psoas.	Sig. Test (W. Prec. Group).
No.	Class.					
1.....	Birth.....	10	Cm. 0.32	—	Cm. 0.41	—
2.....	Gram. 100	5	0.33	N.S.	0.43	N.S.
3.....	150	5	0.41	X	0.52	N.S.
4.....	220	5	0.49	X	0.60	N.S.
5.....	320	5	0.61	XX	0.78	N.S.
6.....	480	5	0.79	XX	0.95	N.S.
7.....	600	10	0.99	XX	0.99	N.S.
8.....	1200	10	1.30	XX	1.55	XX
9.....	1800	10	1.57	XX	1.96	XX
10.....	2400	10	1.64	XX	2.10	X
11.....	3000	10	1.78	XX	2.28	XX
12.....	Mature....	10	1.82	N.S.	2.34	N.S.

Along its length, M. Psoas major takes origin from the body of the last thoracic vertebra, and all lumbar vertebrae, as well as from the transverse processes of the last-named. As the muscle must be damaged along its medial margin during removal, it is evident that width will be influenced by the degree of damage inflicted in different animals. Furthermore, in order to obtain an individual unit, it was deemed advisable to make a separation where the terminal portion of M. Psoas major joins with M. Iliacus,

TABLE 12.
Width of Gastrocnemius muscle.

Site in Muscle.	Burr.			100 Gm.			150 Gm.			220 Gm.			320 Gm.			480 Gm.		
	Significance Test.		Width Cm.	Significance Test.		Width Cm.	Significance Test.		Width Cm.	Significance Test.		Width Cm.	Significance Test.		Width Cm.	Significance Test.		Width Cm.
	Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.	
A.....	—	—	0.26	—	—	0.28	—	—	0.29	—	—	0.37	—	—	0.47	—	—	0.58
B.....	XX	XX	0.37	XX	XX	0.36	XX	XX	0.48	XX	XX	0.58	XX	XX	0.72	XX	XX	0.81
C.....	XX	XX	0.39	N.S.	XX	0.39	XX	XX	0.49	X	XX	0.63	XX	XX	0.78	XX	XX	1.04
D.....	XX	XX	0.34	XX	XX	0.34	X	XX	0.45	X	XX	0.53	XX	XX	0.65	XX	XX	0.87
E.....	XX	XX	0.22	XX	XX	0.25	XX	N.S.	0.34	XX	XX	0.35	N.S.	XX	0.43	XX	XX	N.S.

Site in Muscle.	600 Gm.			1200 Gm.			1800 Gm.			2400 Gm.			3000 Gm.			"Mature".		
	Significance Test.		Width Cm.	Significance Test.		Width Cm.	Significance Test.		Width Cm.	Significance Test.		Width Cm.	Significance Test.		Width Cm.	Significance Test.		Width Cm.
	Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.	
A.....	—	—	0.77	—	—	0.96	—	—	1.16	—	—	1.23	—	—	1.32	—	—	1.36
B.....	XX	XX	1.15	XX	XX	1.61	XX	XX	1.79	XX	XX	1.87	XX	XX	2.03	XX	XX	2.06
C.....	XX	XX	1.23	XX	XX	1.69	X	XX	1.91	XX	XX	1.98	XX	XX	2.16	XX	XX	2.21
D.....	XX	XX	1.06	XX	XX	1.41	XX	XX	1.73	XX	XX	1.83	XX	XX	2.00	XX	XX	2.08
E.....	XX	N.S.	0.76	XX	N.S.	1.00	XX	XX	1.25	XX	XX	1.30	XX	N.S.	1.35	XX	N.S.	1.39

not more caudally where a common stout tendon serves as insertion for both muscles. These facts must be taken into consideration in interpreting the data.

In *M. Gastrocnemius*, only in the first group at 100 gm. live-weight and in the final stage in the mature animal is the increment not significant. However, in the *Psoas* muscle, increase in the value of muscle width from group to group is insignificant in all the earlier groups up to 600 gm. live-weight, as well as in the mature animals. Perhaps the factors mentioned above are partly responsible. In general, the muscles widen until 3,000 gm. live-weight, but over the final six months of the lifetime of the experimental animals (i.e., 3,000 gm. to maturity 3,072 gm.), no widening can be demonstrated.

Apart from width as a general measure, it is of interest to consider the relationship between width at any site along the length of the muscle, relative to the other sites studied.

M. Gastrocnemius.

In Table 12 and Figure 8 are given details of width within *M. Gastrocnemius*. The muscle shows a distinct belly, as width at mid-points C, B, and D, is always greater than towards the origin (A) and insertion (E). Except at birth and 100 gm. live-weight, the middle of the muscle (C) is the significantly widest portion throughout.

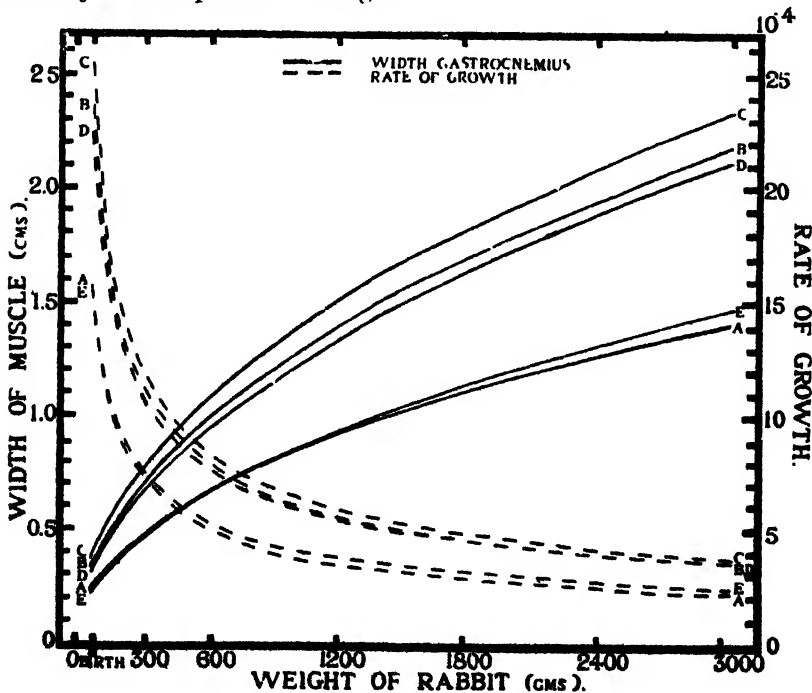


Fig. 8.—Width of muscle.

In Table 13 and Figure 8 are to be found details concerning rate of growth. Always greatest at birth, and corresponding to absolute width in order C, B, D, E, A, the curves descend steeply until 320 gm. live-weight,

thereafter flattening and becoming nearly horizontal from 1,800 gm. A clear picture is presented of a greater growth force in mid-muscle producing larger increments of width increase as the animal grows, than at both ends of the muscle.

TABLE 13.
Rate of growth of muscle width.

Group.	M. Gastrocnemius.				
	Site A.	Site B.	Site C.	Site D.	Site E.
Birth.....	·001,575	·002,367	·002,547	·002,247	·001,563
100 gm.....	·001,307	·001,978	·002,124	·001,887	·001,312
150 gm.....	·001,064	·001,622	·001,739	·001,556	·001,082
220 gm.....	·000,879	·001,348	·001,444	·001,300	·000,904
320 gm.....	·000,711	·001,099	·001,175	·001,066	·000,741
480 gm.....	·000,576	·000,897	·000,958	·000,875	·000,608
600 gm.....	·000,501	·000,784	·000,836	·000,768	·000,534
1200 gm.....	·000,355	·000,562	·000,597	·000,555	·000,386
1800 gm.....	·000,289	·000,461	·000,489	·000,458	·000,318
2400 gm.....	·000,248	·000,397	·000,421	·000,396	·000,275
3000 gm.....	·000,222	·000,357	·000,378	·000,358	·000,248
Mature.....	·000,220	·000,354	·000,374	·000,354	·000,246

Group.	M. Psoas.				
	Site A.	Site B.	Site C.	Site D.	Site E.
Birth.....	·002,060	·002,459	·002,712	·002,779	·002,665
100 gm.....	·001,716	·002,051	·002,265	·002,322	·002,213
150 gm.....	·001,410	·001,679	·001,857	·001,904	·001,803
220 gm.....	·001,175	·001,394	·001,543	·001,583	·001,491
320 gm.....	·000,960	·001,135	·001,258	·001,291	·001,208
480 gm.....	·000,785	·000,925	·001,027	·001,054	·000,979
600 gm.....	·000,687	·000,808	·000,897	·000,921	·000,852
1200 gm.....	·000,494	·000,577	·000,642	·000,660	·000,604
1800 gm.....	·000,406	·000,473	·000,528	·000,541	·000,492
2400 gm.....	·000,350	·000,407	·000,454	·000,466	·000,422
3000 gm.....	·000,316	·000,366	·000,408	·000,420	·000,379
Mature.....	·000,313	·000,362	·000,404	·000,415	·000,375

M. Psoas.

Width of this muscle is considered in Table 14 and Figure 9.

Belly formation is not as pronounced as in *M. Gastrocnemius*, though width at D and C is always greater than points E, B, A, in order of decreasing magnitude. No doubt the value for E would be smaller, to approximate width at A, if the common muscle termination had been severed more caudally.

In general, the muscle widens from A to B to C to D, but narrows from D to E. With two possible exceptions, it is shown that the muscle is always significantly narrowest at site A (near the origin).

Rate of growth (Table 13 and Figure 9) bears a close resemblance to that of *M. Gastrocnemius*, but the rate is higher throughout for the *Psoas* muscle. Growth rate is greatest at birth, then the curve falls almost vertically until 320 gm. live-weight. From this stage it drops more gradually until 1,800 gm. live-weight to assume a nearly horizontal level for the final two groups. As in *M. Gastrocnemius*, the rate of growth is highest about the middle of the muscle (D, C). Towards the ends the growth rate is lower, least of all near the muscle origin at A.

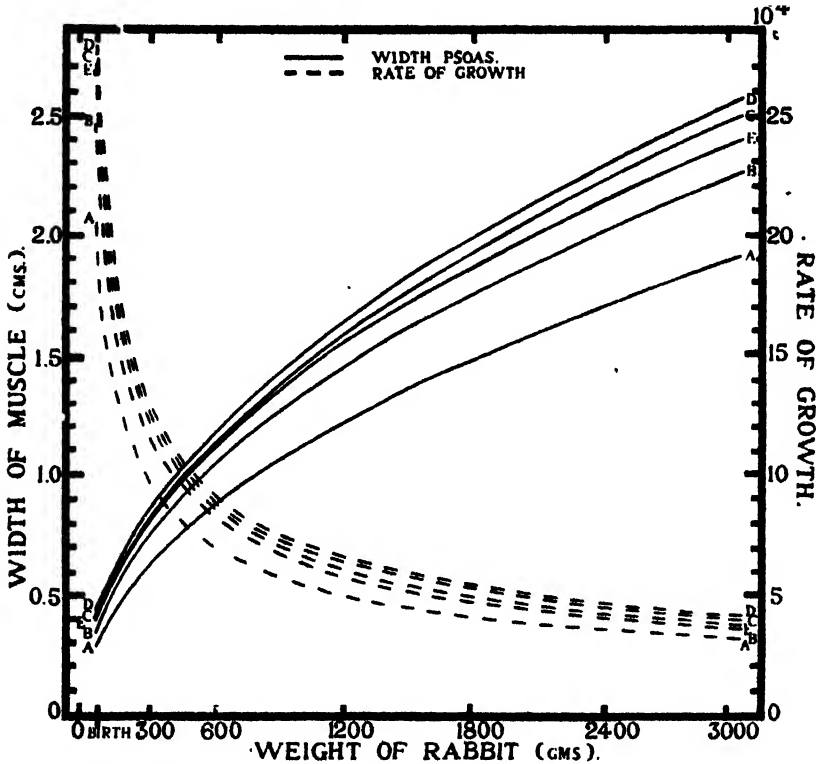


Fig. 9.—Width of *M. Psoas*.

Discussion.

In the *Longissimus dorsi* muscle of various domestic animals, it has been inferred that width and depth increase after length has become stabilised. In this experiment muscle length has been shown to increase up to a live-weight of 3,000 gm., yet no widening of muscle has been demonstrated in the six months' period which elapsed after this stage. Explanation is complicated by view of the fact that different species, as well as different muscles, are involved. For example, it is not possible to assess the developmental age of the rabbits studied in terms of the corresponding period, of say the sheep or pig.

Clearly, the structural arrangement (and form) of a muscle is largely dependent on the function the muscle is required to perform in the animal body. Why is *M. Gastrocnemius* relatively short, with a pronounced belly, as compared with the long *Psoas* muscle, which has a slightly more discreet spreading about its middle? More information is required regarding the

nature of muscular contractility in the different muscles. Obviously a wide field is open for investigation of muscular function and efficiency, as related to form and structural composition.

4. Depth.

(Literature: Page 339.)

The next dimension to be considered is depth (or thickness).

TABLE 15.

Depth of muscle.

GROUPS OF RABBITS.		No. of Rabbits.	Mean Depth M. Gastrocn.	Sig. Test (W. Prec. Group).	Mean Depth M. Psoas).	Sig. Test (W. Prec. Group).
No.	Class.					
1.....	Birth.....	10	Cm. 0.11	—	Cm. 0.06	—
2.....	Gram. 100	5	0.12	N.S.	0.06	N.S.
3.....	150	5	0.13	N.S.	0.07	N.S.
4.....	220	5	0.16	X	0.08	N.S.
5.....	320	5	0.20	XX	0.11	N.S.
6.....	480	5	0.26	XX	0.14	N.S.
7.....	600	10	0.31	XX	0.15	N.S.
8.....	1200	10	0.35	XX	0.24	XX
9.....	1800	10	0.40	XX	0.30	XX
10.....	2400	10	0.43	XX	0.33	XX
11.....	3000	10	0.44	N.S.	0.34	N.S.
12.....	Mature....	10	0.42	X	0.36	N.S.

In Table 15 are to be found details concerning muscle depth, considered as a measure of the mean value of five sites within each muscle.

In M. Gastrocnemius, the increment is significant for each succeeding group from 150 gm. live-weight until 2,400 gm. At 3,000 gm. live-weight the increase is not significant, hence it may be a chance variation. It is, however, puzzling to find a significant decrease from 3,000 gm. live-weight to maturity. This positive result when group 12 is tested against group 11 may be due to the accident of a low figure in the final group. When this variation in depth (0.02 cm.) is taken into consideration, it will readily be appreciated how slight need be an error in measurement to produce this apparent contradiction. Hirzel (1936) found an actual decrease in depth in some heavier classes of sheep. His observations that animals packing on fat very rapidly reach a high weight due to fat growth, as distinct from muscle growth, may have some bearing on the anomaly observed.

Mean depth of the Psoas muscle shows an increase which is, however, distributed unevenly over the lifetime of the rabbits studied. The increase becomes significant only when a live weight of 1,200 gm. has been achieved and the increments at both 3,000 gm. live-weight and maturity are again insignificant.

M. Gastrocnemius.

With regard to the variations in depth within this muscle, Table 16 and Figure 10 present a picture of a more or less uniformly deep muscle which tapers markedly towards its insertion. E is always the shallowest portion of

TABLE 16.
Depth of Gastrocnemius muscle.

Site in Muscle.	BIRTH.			100 Gm.			150 Gm.			200 Gm.			250 Gm.			480 Gm.		
	Significance Test.		Depth Cm.	Significance Test.		Depth Cm.	Significance Test.		Depth Cm.	Significance Test.		Depth Cm.	Significance Test.		Depth Cm.	Significance Test.		Depth Cm.
	Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.	
A.....	—	—	0.10	—	—	0.11	—	—	0.13	—	—	0.17	—	—	0.22	—	—	—
B.....	XX	XX	0.12	XX	XX	0.15	XX	XX	0.20	XX	XX	0.25	XX	XX	0.31	XX	XX	XX
C.....	XX	XX	0.13	XX	XX	0.14	N.S.	XX	0.21	N.S.	XX	0.25	N.S.	XX	0.32	N.S.	XX	XX
D.....	XX	XX	0.12	XX	XX	0.12	N.S.	XX	0.16	XX	N.S.	0.21	XX	XX	0.28	XX	XX	XX
E.....	XX	XX	0.08	XX	XX	0.08	XX	X	0.09	XX	XX	0.10	XX	XX	0.15	XX	XX	XX
<hr/>																		
Site in Muscle.	600 Gm.			1200 Gm.			1800 Gm.			2400 Gm.			3000 Gm.			"MAYTAG."		
	Significance Test.		Depth Cm.	Significance Test.		Depth Cm.	Significance Test.		Depth Cm.	Significance Test.		Depth Cm.	Significance Test.		Depth Cm.	Significance Test.		Depth Cm.
	Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.		Preceding Site.	Site A.	
.....	—	—	0.32	—	—	0.39	—	—	0.42	—	—	0.44	—	—	0.45	—	—	—
.....	XX	XX	0.35	XX	XX	0.36	N.S.	N.S.	0.42	N.S.	N.S.	0.45	N.S.	N.S.	0.44	N.S.	N.S.	N.S.
.....	XX	XX	0.33	XX	N.S.	0.37	N.S.	X	0.44	XX	XX	0.47	N.S.	XX	0.48	X	N.S.	X
.....	N.S.	N.S.	0.33	N.S.	XX	0.36	N.S.	XX	0.42	XX	N.S.	0.46	N.S.	N.S.	0.49	N.S.	X	XX
.....	XX	XX	0.20	XX	XX	0.23	XX	XX	0.28	XX	XX	0.30	XX	XX	0.34	XX	XX	XX

TABLE 18.
Depth of Psoas muscle.

Site in Muscle.	BIRTH.				100 Gm.				150 Gm.				220 Gm.				320 Gm.				480 Gm.			
	Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.	
	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.
A.....	0.03	—	—	0.03	—	—	0.03	—	—	0.03	—	—	0.04	—	—	0.06	—	—	0.07	—	—	—	—	—
B.....	0.06	XX	XX	0.04	XX	XX	0.06	XX	XX	0.06	XX	XX	0.06	XX	XX	0.08	XX	XX	0.11	XX	XX	0.11	XX	XX
C.....	0.06	XX	XX	0.06	XX	XX	0.06	XX	XX	0.08	XX	XX	0.09	XX	XX	0.10	XX	XX	0.14	XX	XX	0.14	XX	XX
D.....	0.07	XX	XX	0.07	XX	XX	0.09	XX	XX	0.09	XX	XX	0.11	XX	XX	0.13	XX	XX	0.18	XX	XX	0.18	XX	XX
E.....	0.09	XX	XX	0.09	XX	XX	0.11	XX	XX	0.11	XX	XX	0.12	XX	XX	0.17	XX	XX	0.21	XX	XX	0.21	XX	XX

Site in Muscle.	600 Gm.				1200 Gm.				1800 Gm.				2400 Gm.				3000 Gm.				"MATURE".			
	Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.		Significance Test.	
	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.	Depth Cm.	Preceding Site.	Site A.
A.....	0.06	—	—	0.15	—	—	0.19	—	—	0.19	—	—	0.20	—	—	0.22	—	—	0.24	—	—	—	—	—
B.....	0.12	XX	XX	0.16	XX	XX	0.25	XX	XX	0.25	XX	XX	0.27	XX	XX	0.28	XX	XX	0.29	XX	XX	0.29	XX	XX
C.....	0.16	XX	XX	0.24	XX	XX	0.31	XX	XX	0.31	XX	XX	0.34	XX	XX	0.34	XX	XX	0.37	XX	XX	0.37	XX	XX
D.....	0.18	XX	XX	0.27	XX	XX	0.34	XX	XX	0.34	XX	XX	0.40	XX	XX	0.40	XX	XX	0.42	XX	XX	0.42	XX	XX
E.....	0.21	XX	XX	0.33	XX	XX	0.40	XX	XX	0.40	XX	XX	0.47	XX	XX	0.48	XX	XX	0.50	XX	XX	0.50	XX	XX

the muscle. Significance is present throughout, from birth to maturity. Depth in the middle of the muscle (C and B) is greater than towards the ends (D and A) until 1,800 gm. live-weight. At this stage the initially steep curves for C and B flatten abruptly to cross under those for D and A. As a result *M. Gastrocnemius* is deepest at D and A respectively, in the final group.

These changes are explained by a study of the rate of growth (Table 17, Figure 10). The growth rate is greatest at birth, for all the sites, A to E. Although the curves at B and C fall almost vertically until 480 gm. live-weight, they do not subsequently flatten to the same degree as those for D, A, and E, consequently cross over them to assume a lower level. At birth, there is a striking difference between the rate of growth at the highest point B and the lowest point E, but the difference is very slight in the final groups, when the growth impetus is nearly played out.

TABLE 17.

Rate of growth of muscle depth.

Group.	M. Gastrocnemius.				
	Site A.	Site B.	Site C.	Site D.	Site E.
Birth.....	·000,583	·001,035	·000,977	·000,635	·000,403
100 gm.....	·000,476	·000,835	·000,776	·000,514	·000,329
150 gm.....	·000,382	·000,637	·000,589	·000,407	·000,264
220 gm.....	·000,311	·000,479	·000,446	·000,328	·000,214
320 gm.....	·000,247	·000,334	·000,318	·000,258	·000,171
480 gm.....	·000,197	·000,222	·000,220	·000,204	·000,136
600 gm.....	·000,170	·000,164	·000,169	·000,174	·000,117
1200 gm.....	·000,117	·000,067	·000,082	·000,118	·000,080
1800 gm.....	·000,094	·000,034	·000,050	·000,093	·000,064
2400 gm.....	·000,079	·000,018	·000,033	·000,078	·000,054
3000 gm.....	·000,070	·000,010	·000,024	·000,069	·000,048
Mature.....	·000,070	·000,009	·000,023	·000,069	·000,048

Group.	M. Psoas.				
	Site A.	Site B.	Site C.	Site D.	Site E.
Birth.....	·000,201	·000,293	·000,381	·000,447	·000,542
100 gm.....	·000,174	·000,249	·000,321	·000,374	·000,452
150 gm.....	·000,149	·000,208	·000,265	·000,308	·000,370
220 gm.....	·000,129	·000,175	·000,222	·000,256	·000,308
320 gm.....	·000,109	·000,145	·000,183	·000,209	·000,250
480 gm.....	·000,093	·000,121	·000,150	·000,171	·000,204
600 gm.....	·000,084	·000,107	·000,132	·000,150	·000,178
1200 gm.....	·000,064	·000,079	·000,096	·000,108	·000,128
1800 gm.....	·000,055	·000,065	·000,079	·000,088	·000,104
2400 gm.....	·000,049	·000,057	·000,069	·000,076	·000,090
3000 gm.....	·000,045	·000,052	·000,062	·000,069	·000,082
Mature.....	·000,045	·000,051	·000,061	·000,068	·000,080

MEAT STUDIES I.—POST-NATAL GROWTH AND DEVELOPMENT OF MUSCLE.

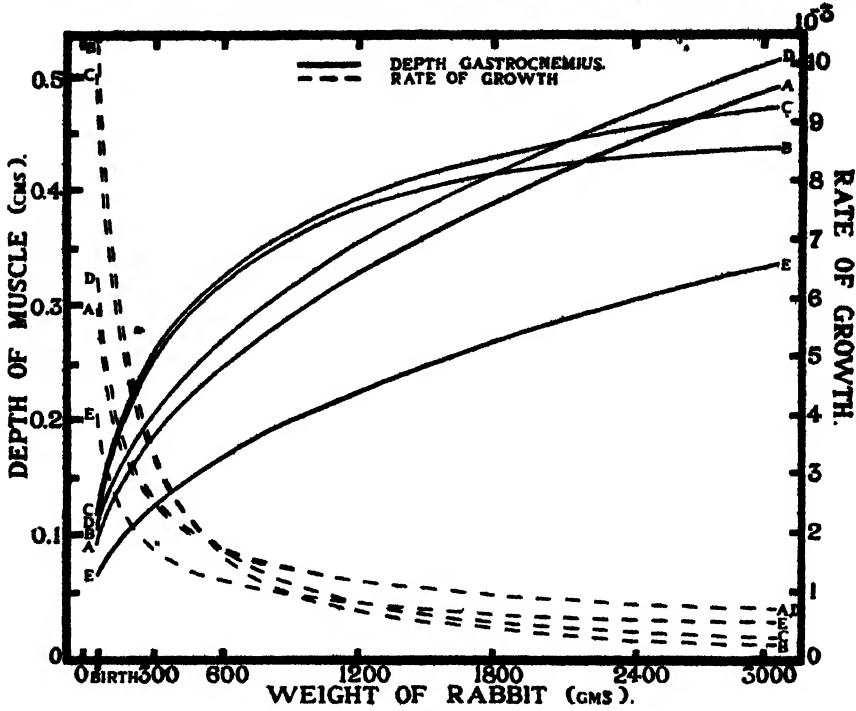


Fig. 10.—Depth of M. Gastrocnemius.

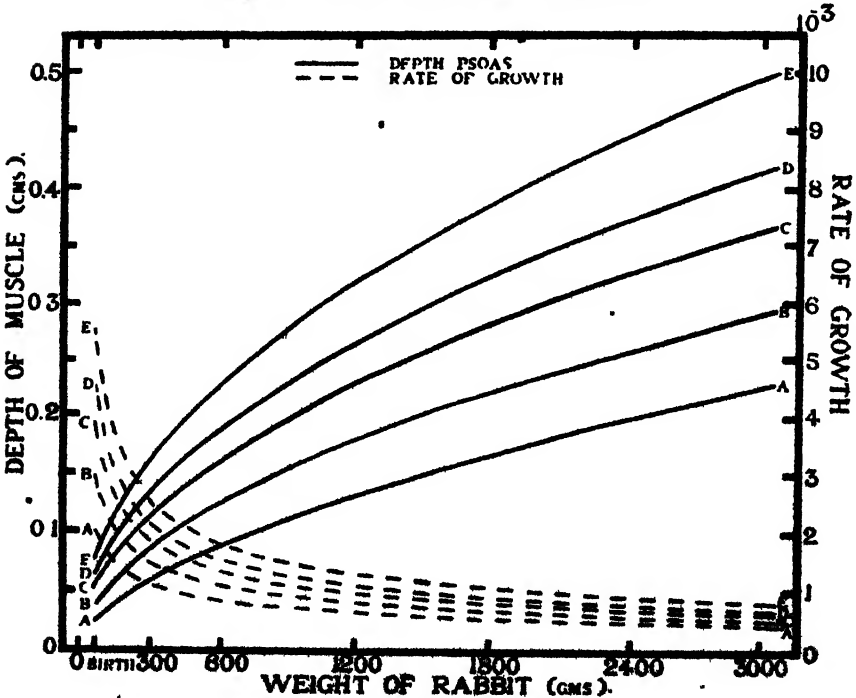


Fig. 11.—Depth of M. Psoas.

M. Psoas.

Depth of the Psoas muscle is considered in Table 18 and Figure 11. In contradistinction to *M. Gastrocnemius*, *M. Psoas* is thinnest near its origin (A) and thickens uniformly along its length, so that it is always thickest towards the insertion (E). When the measurements at each site are compared, all the variations are found to be significant for each group from birth to maturity.

Interpreted in terms of growth rate (Table 17. and Figure 11), the thickest portions of the muscle grow fastest throughout—in order E, D, C, B, A, respectively. Rate of increase is greatest at birth, falls rapidly until 220 gm. live-weight, then subsides more gradually to become almost stationary from 1,200 gm. live-weight onwards. When compared with the data for *M. Gastrocnemius*, rate of growth of Psoas is found to be initially less, yet it does not decrease to the same degree in the final stages. Whereas the early developing *Gastrocnemius* muscle has a relatively high rate of growth early in life, the later maturing Psoas muscle maintains an initially lower growth impetus over later stages of life.

Discussion.

In neither muscle has it been possible to show an increase in depth from 2,400 gm. live-weight to 3,000 gm., nor in the six months' period subsequent to the latter stage. From the data presented, the conclusion is drawn that depth of muscle has become stable even before length and width. Yet Hammond (1936) and McMeekan (1940-41) showed that, in the ox, sheep and pig, *M. Longissimus dorsi* increases in depth after the cessation of widening of the muscle. Is it possible a different mechanism regulates the inter-relationship in different muscles, and in different species?

Another question that arises is the reason for the varying shape of the two muscles studied. Whereas the Psoas muscle is thickest near the insertion and tapers uniformly to become thin and sheet-like at its origin, *M. Gastrocnemius* is relatively thick from its origin along the greater part of the muscle, only tapering out suddenly near its insertion. Speculation is unlikely to be profitable in the absence of knowledge concerning the nature of the contractile process, its speed, and duration.

5. *The inter-relation of weight, length, width, and depth of muscle.**Discussion.*

A diagrammatic representation of the changes in growing muscle is shown in Figure 12. Length seems to be the major factor in determining mass of muscle, not only by virtue of its relatively greater magnitude, but also because the effect of increasing width and depth is automatically increased as the muscle becomes longer.

The weight and the dimensions considered are by no means proportional at the various stages. Weight increases to a markedly greater degree than length, width, and depth, especially from about 600 gm. live-weight onwards. However, not only shape but also the composition of muscle determines weight, as models of identical form but of different composition (e.g., tin, lead) are obviously of dissimilar weight. Hence weight cannot be related to shape unless the changing chemical composition and density are also known. Herein lies a difficulty in assessing the relative importance of length, width, and depth, in terms of increasing weight of muscle.

Although the chemical composition of muscle has not been considered in this study it is well known that muscle changes in composition as an animal becomes older. For instance, Hammond (1932) found a decrease in the percentage of water, a slight increase of protein and ash, and a marked increase of fat. A knowledge of these variations is essential for evaluating the relative importance of the dimensional changes in effecting the increase in muscle mass. Nevertheless, it is of interest to discuss the relative changes of these dimensions in terms of the general order of development in post-natal life (Table 19, Figure 13).

TABLE 19.

Relative growth, as percentage of measurement at birth.

Group.	Gastrocnemius Muscle.				Psoas Muscle.			
	Weight.	Length.	Width.	Depth.	Weight.	Length.	Width.	Depth.
Birth.....	100	100	100	100	100	100	100	100
100 gm.....	117	109	103	107	143	132	105	97
150 gm.....	244	153	128	118	246	158	128	123
220 gm.....	471	206	156	147	395	190	148	140
320 gm.....	883	253	193	178	798	243	193	180
480 gm.....	1,556	303	252	233	1,435	296	235	237
600 gm.....	2,000	330	309	282	1,773	324	248	250
1,200 gm.....	3,800	410	406	318	4,182	375	388	400
1,800 gm.....	5,900	500	491	364	7,818	460	490	500
2,400 gm.....	7,420	550	513	382	10,500	533	525	550
3,000 gm.....	9,280	590	556	400	13,446	581	570	567
Mature.....	9,440	620	569	382	14,664	588	585	600

That weight develops to a much greater extent than length, width, or depth, is clearly indicated. Reference to Table 19 reveals how this divergence becomes increasingly pronounced as the animal ages. As the Psoas muscle has previously been shown to have a later period of growth than M. Gastrocnemius, it is not surprising that this same trend is again revealed. Thus, in the mature rabbit, the relative weight of the Psoas muscle has increased roughly one and a half times as much as that for M. Gastrocnemius.

In both muscles the length growth surpasses both width and depth up to 600 gm. live-weight. This conforms to expectation, but it is surprising that this supremacy is maintained in the Gastrocnemius muscle throughout the lifetime of the animal. Even in the final stages length is still on the up-grade. In this muscle the trend for width and depth is similar in the initial groups, but from 600 gm. live-weight depth increases at a lesser rate than width. There is an increasing divergence as the animal grows, so that in the mature rabbit the curve for depth lies far below that for width. Thus a gradient exists in the Gastrocnemius muscle as regards the order of development. Firstly, developing earliest and most is the growth in length, next width, and lastly depth.

In the Psoas muscle, growth in length, width, and depth up to 600 gm. live-weight behave similarly to M. Gastrocnemius. However, in the succeeding groups, the respective curves cross and re-cross. Apparently the order of development of the various dimensions closely resemble each other after a body weight of 600 gm. has been attained.

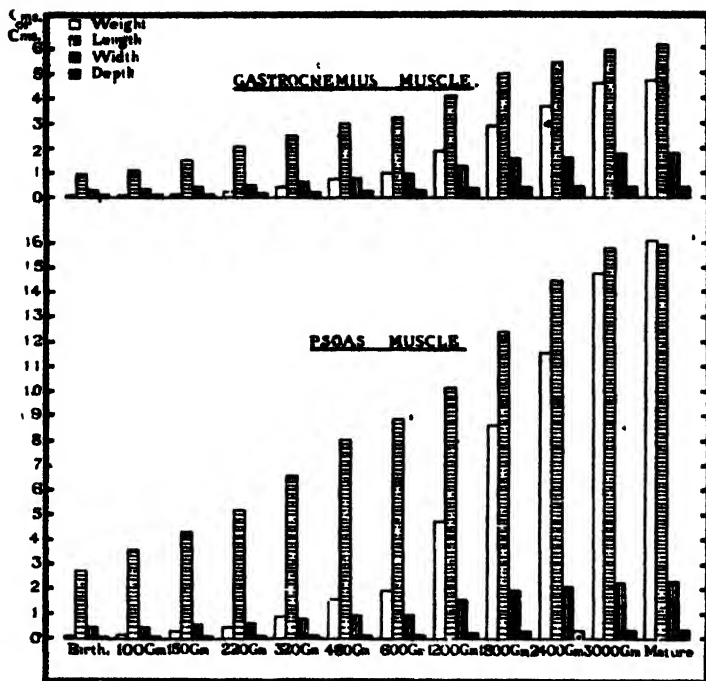


Fig. 12.—Changes in mass and dimensions of muscle.

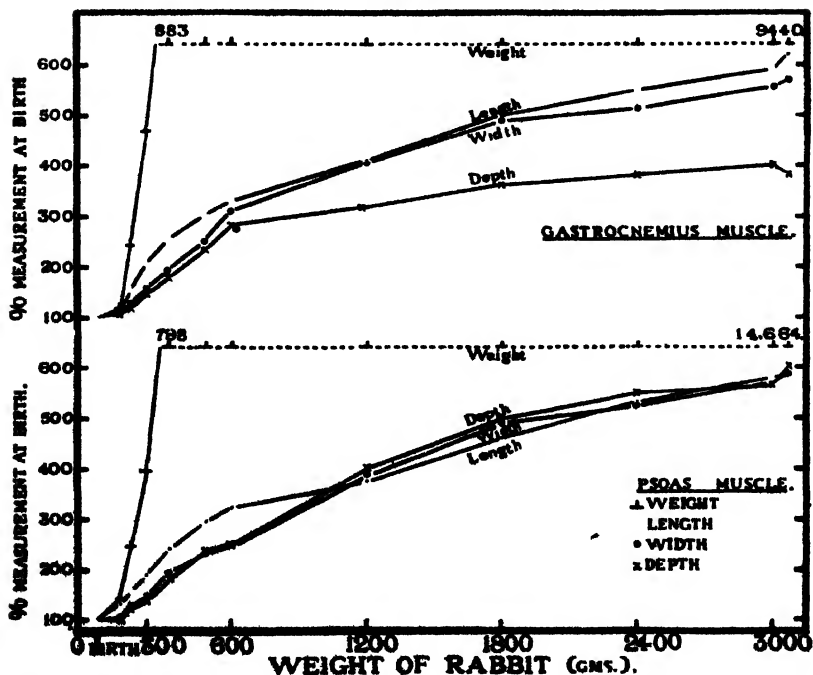


Fig. 13.—Relative growth, as percentage of measurement at birth.

This comparison shows that the order of development varies in different muscles. Inherent structural differences such as those previously described, together with the resulting variation in the respective growth mechanism, make it necessary to treat each muscle on its merits. Hence it is unwise to make assumptions, in terms of muscle in general, from this limited study. Before this is possible it will be necessary to carry out a more detailed investigation of skeletal musculature, of widely varying structure and function

(c) GROWTH AND DEVELOPMENT OF MUSCLE BUNDLE.

(Literature pages 339-341).

Having demonstrated changes in morphology of the growing muscle unit, as a whole, one may now consider the constituent units of muscle tissue. As far as is possible, morphological changes in the muscle bundle have been studied in similar manner to the individual muscle.

1. *Length of Muscle Bundle.*

M. Gastrocnemius.

Length of fasciculus is considered in Table 20 and Figure 14.

TABLE 20.

Length of Bundle—M. Gastrocnemius.

GROUPS OF RABBITS.		No. of Rabbits.	Mean Bundle Length.	SIGNIFICANCE TEST.	
No.	Class.			(W. Prec. Group).	(W. Group 4).
	Gm.		Cm.		
4.....	220	5	0.73	—	—
5.....	320	5	0.95	XX	XX
6.....	480	5	1.01	N.S.	XX
7.....	600	10	0.93	N.S.	XX
8.....	1,200	10	0.90	N.S.	XX
9.....	1,800	10	1.00	XX	XX
10.....	2,400	10	0.99	N.S.	XX
11.....	3,000	10	0.94	N.S.	XX
12.....	Mature.....	10	0.97	N.S.	XX

It was not possible to determine the changes in length that occur in the early stages of life (up to 150 gm. live-weight). Dissection of these filamentous short bundles is difficult. Even though the bundles are handled carefully, they break easily because they are so frail. Probably a sectioning method may facilitate measurement, provided precautions are taken to ensure the sectioned bundles are straight in the finished preparations.

It is seen that there is a definite increase in length from 220 gm. live-weight to 320 gm. Although there is no detectable increase in the groups following, bundle length in these succeeding groups is always greater than for the 220 gm. group. A peculiar feature is seen in group 9 where there is a significant lengthening as compared with group 8. This is undoubtedly due to the accident of a low figure at 1,200 gm. live-weight together with a high figure at 1800 gm. Under the circumstances it can be said that the bundle lengthens between 220 and 320 gm. live-weight after which no further increase in length takes place.

As the length of the Gastrocnemius bundle was not determined for the first three groups, it is not possible to calculate the rate of growth from birth onwards. However, these changes in growth rate of bundle length may be deduced from a study of the foregoing data. Although growth must be extremely active in the initial groups, it loses its impetus relatively early in life (320 gm. live weight). Presumably, after a high initial rate of growth at birth, the rate of lengthening must decrease markedly to become stationary from 320 gm. to maturity. Such a picture is not at variance with the view that the bundle rapidly lengthens at birth and shortly afterwards, until achieving stability at 320 gm. live-weight.

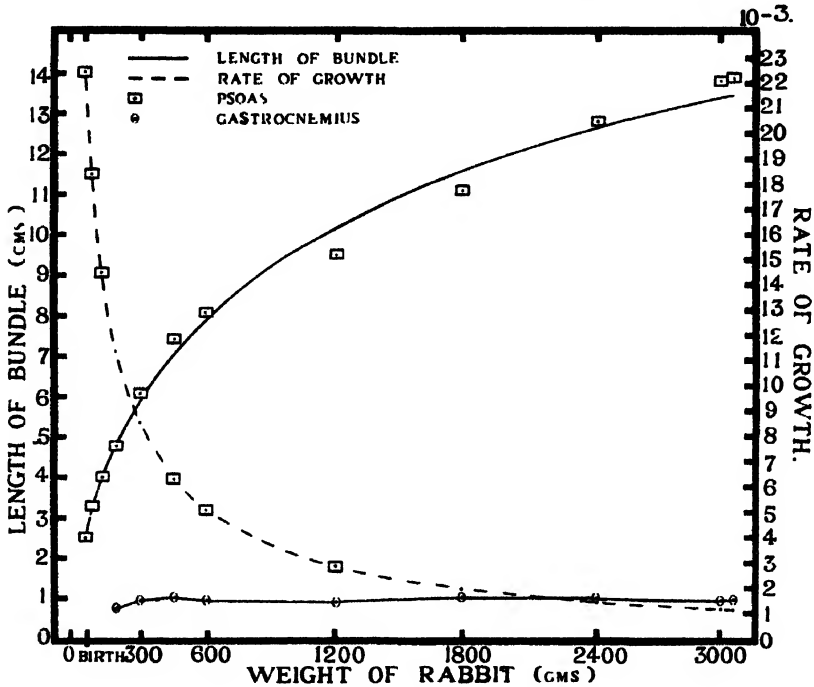


Fig 14.—Length of muscle bundle.

The lengths tabulated in Table 21 are those of fasciculi at sites A, B, C, D, E, along the length of the muscle. In Figure 15, a graphical representation gives a picture of the mean bundle length at the various sites. For reasons which will soon be evident, three curves are compiled from the data, for the groups from 600 gm. live-weight to maturity, from 320 to 480 gm., and at 220 gm. live-weight.

At 220 gm. live-weight there is a tendency for a progressive increase in length from A to E i.e. the shortest bundles occur at A. Although there is the same general trend at 320 gm. live-weight, and at 480 gm., no significant difference can be shown between sites A and B. For all the subsequent groups, however, B is in each case shorter than A, with a progressive increase in length at the succeeding points C, D and E. Here, as in the earlier groups, the bundles nearer the insertion are always longer than those nearer the origin of the muscle, but the shortest bundles are always

TABLE 21.
Length of Bundle—Gastrocnemius Muscle.

Site in Muscle.	220 Gm.			320 Gm.			480 Gm.			600 Gm.			1,200 Gm.			1,800 Gm.			2,400 Gm.			3,000 Gm.			' Mature "		
	Sig. T.			Sig. T.			Sig. T.			Sig. T.			Sig. T.			Sig. T.			Sig. T.			Sig. T.			Sig. T.		
	L.	Cm.	P.	L.	Cm.	P.	L.	Cm.	P.	L.	Cm.	P.	L.	Cm.	P.	L.	Cm.	P.	L.	Cm.	P.	L.	Cm.	P.	L.	Cm.	P.
A.....	0.68	—	—	0.91	—	—	0.91	—	—	0.91	—	—	0.91	—	—	0.91	—	—	0.91	—	—	0.91	—	—	0.91	—	—
B.....	0.72	X	X	0.91	NS	0.98	NS	0.98	NS	0.98	X	X	0.84	X	X	0.93	XX	XX	0.92	XX	XX	0.86	XX	XX	0.91	XX	XX
C.....	0.75	X	XX	0.95	XX	XX	1.00	NS	NS	0.92	XX	NS	0.86	NS	NS	0.97	X	NS	0.94	NS	X	0.92	XX	XX	0.95	XX	NS
D.....	0.76	NS	XX	0.97	X	XX	1.02	NS	XX	0.96	XX	XX	0.91	NS	NS	1.03	XX	X	1.02	XX	X	0.98	XX	NS	1.00	XX	X
E.....	0.76	NS	XX	0.99	X	XX	1.05	X	XX	0.99	X	XX	0.97	X	X	1.09	XX	XX	1.08	XX	XX	0.98	NS	NS	1.04	XX	XX

ABBREVIATIONS:—

L. Length.
Sig. T. Significance Test.
P. S. Preceding Site.
S. A. Site A.

found at B. Probably there is a change in the relative length of the bundles at sites A and B in older as opposed to younger animals. Confirmation of this fact may be obtained, if a technique can be devised for measuring the bundles in very small animals, which are not represented in these data.

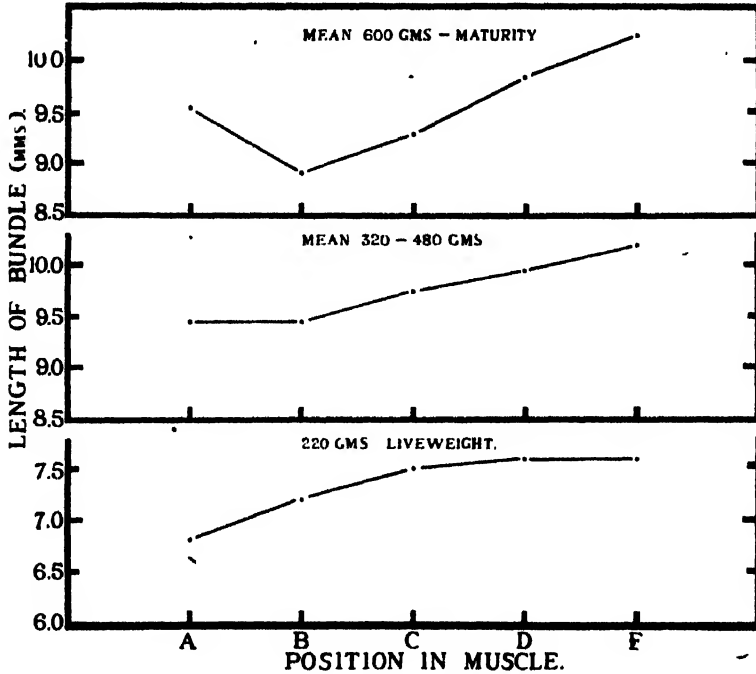


Fig. 15.—Length of bundle, M. Gastrocnemius.

Angle of Bundle in M. Gastrocnemius.

During dissection it was observed that the angle of bundles to the long axis of the Gastrocnemius muscle varies appreciably from the earlier to later groups. A method is available for calculating this angle, namely, the mathematical formulation that $\text{Sine of angle} = \frac{\text{Side opposite the angle.}}{\text{Hypotenuse.}}$

Applying this formulation to the muscle, it follows that Sine of angle of bundle to long axis of M. Gastrocnemius equals—

$$\frac{\text{Depth of muscle.}}{\text{Length of bundle.}}$$

In Table 22 are shown the respective values of this angle from 220 gm. live-weight onwards. In the young animal the fasciculi are steeply inclined in relation to the long axis of the muscle, but as the animal grows the bundles assume a progressively more obtuse relationship to the long axis. This is shown graphically in a diagrammatic form in Figure 16. The apparent reversal of trend in the mature animal is the possible result of an error or abnormality. Probably the measurement of the depth of muscle for this group is implicated, as the same anomaly is there apparent.

TABLE 22.

Angle of Bundle to Long Axis of M. Gastrocnemius.

GROUPS OF RABBITS.		Number of Rabbits.	Mean Angle Sites A-E. (Degrees).
No.	Class.		
4.....	220 gm.....	5	12.8
5.....	320 gm.....	5	12.2
6.....	480 gm.....	5	14.8
7.....	600 gm.....	10	19.4
8.....	1,200 gm.....	10	22.9
9.....	1,800 gm.....	10	23.6
10.....	2,400 gm.....	10	25.9
11.....	3,000 gm.....	10	28.1
12.....	Mature.....	10	25.7

Apart from these general changes in the relative position of the Gastrocnemius bundles, a well-defined gradient exists between the fascicular angles at varying sites within the muscle (Table 23). For all the groups, the angle is slight near the origin at site A, but it becomes larger along the muscle to reach a maximum value at mid-muscle (B or C). Towards the muscle insertion the angle again decreases from C to D to E, so much so that the bundles at the terminal site are more nearly longitudinally inclined than anywhere else within the muscle.

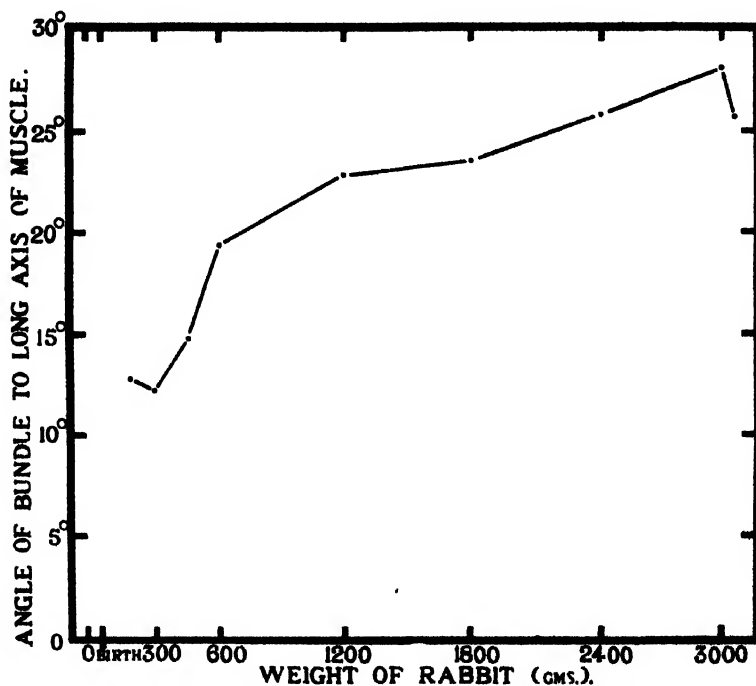


Fig. 16—Angle of bundle to long axis of M. Gastrocnemius.

TABLE 23.

Angle of Bundle to Long Axis of M. Gastrocnemius at Sites A, B, C, D and E.

Site in Muscle.	GROUP.								Mature.
	220 Gm.	320 Gm.	480 Gm.	600 Gm.	1,200 Gm.	1,800 Gm.	2,400 Gm.	3,000 Gm.	
A.....	12.5°	11.0°	13.0°	20.5°	25.0°	25.0°	26.5°	27.5°	25.5°
B.....	16.0°	16.0°	18.5°	23.5°	27.0°	27.0°	29.5°	31.0°	27.0°
C.....	16.5°	15.5°	18.5°	21.5°	25.5°	27.0°	30.0°	31.5°	28.5°
D.....	12.0°	12.5°	16.0°	20.0°	23.5°	24.0°	27.0°	30.0°	28.0°
E.....	7.0°	6.0°	8.0°	11.5°	13.5°	15.0°	16.5°	20.5°	19.5°

M. Psoas.

Table 24 and Figure 14 show that, in contradistinction to *M. Gastrocnemius*, there is a steady increase in bundle length throughout the growth of the animal. Significance is reached at 320 gm. live-weight, but the increment at 600 gm. and at maturity is not significant.

TABLE 24.

Length of Bundle—M. Psoas.

GROUPS OF RABBITS.		No. of Rabbits.	Mean Bundle Length.	SIGNIFICANCE TESTS.	
No.	Class.			(W. Prec. Group).	(W. Group 1).
			Cm.		
1.....	Birth.....	10	2.53	—	—
2.....	100 gm.....	5	3.25	N.S.	N.S.
3.....	150 gm.....	5	4.00	N.S.	XX
4.....	220 gm.....	5	4.78	N.S.	XX
5.....	320 gm.....	5	6.08	X	XX
6.....	480 gm.....	5	7.41	X	XX
7.....	600 gm.....	10	8.12	N.S.	XX
8.....	1,200 gm.....	10	9.51	XX	XX
9.....	1,800 gm.....	10	11.13	XX	XX
10.....	2,400 gm.....	10	12.75	XX	XX
11.....	3,000 gm.....	10	13.83	XX	XX
12.....	Mature.....	10	13.85	N.S.	XX

Details of rate of growth are presented in Table 25 and Figure 14. The rate of length increase is highest at birth, decreases sharply to 220 gm. live-weight, thenceforth more gradually to become almost stationary in the ultimate groups. As is to be expected from the muscle structure, these data closely approximate those dealing with length of *M. Psoas*.

Discussion.

Length of bundle is shown to be an early developing character in *M. Gastrocnemius*. It is remarkable that *Gastrocnemius* bundles achieve their maximum length so early in life (320 gm. live-weight—52 days), with no

MEAT STUDIES I.—POST-NATAL GROWTH AND DEVELOPMENT OF MUSCLE.

further change until maturity (3,072 gm. live-weight—416 days). It is of interest to discuss this finding in relation to muscle length and depth, both of which have been shown to increase until the final stages in the lifetime of the series of animals studied.

TABLE 25.

Rate of growth of length of bundle—M. Psoas.

Group.	Growth Rate.
Birth.....	·022,457
100 gm.....	·018,383
150 gm.....	·014,490
220 gm.....	·011,418
320 gm.....	·008,599
480 gm.....	·006,348
600 gm.....	·005,125
1,200 gm.....	·002,880
1,800 gm.....	·001,979
2,400 gm.....	·001,464
3,000 gm.....	·001,171
Mature.....	·001,145

How does the Gastrocnemius muscle lengthen, if this is not due to lengthening of the component muscle bundles? Over the period from 320 gm. live-weight to maturity this muscle has been shown to lengthen from 2·50 cm. to 6·15 cm., i.e., an increase of 3·65 cm.

By what mechanism do the bundles change their relative position in the growing muscle? The mean fascicular angle changes progressively from 12·2° to 25·7° over the same period. Not only do the bundles maintain constant length, but the changing position of the bundles also tends to reduce their effective length in terms of the long axis of the muscle. An unequal rate of growth of the two aponeuroses to which the ends of the bundles are attached may offer a possible solution. For example, if the aponeurosis of origin (superficial) has a relatively greater growth rate distally along the muscle, the fascicular angle will become more obtuse. At the same time the muscle will be thickened to a degree corresponding with the degree of tilting invoked by this process. Alternatively, if the aponeurosis of insertion (deep) has a relatively greater rate of growth proximally along the muscle the same effect will be produced. Presumably both processes play a part in the growing muscle, the aponeurosis of origin extending down towards the muscle insertion, while the aponeurosis of insertion extends upwards towards the muscle origin.

In general, it appears that the varying position assumed by the bundles as the animal grows will contribute more to thickening of the muscle than to an increase in length. If this is so increase in length of the muscle will be dependent upon a thickening of the bundles, and ultimately of the innumerable fibres comprising these bundles. Contradictory as it may seem, lengthening of the muscle is apparently due to a thickening of the bundles (or fibres), whereas thickening of the muscle is at least partly due to an apparent lengthening of the bundles, as the result of a change in their relative position.

In *M. Psoas*, where a different process is at work, increase in muscle length can be attributed to a straightforward lengthening of the individual fasciculi. Increase in the depth of this muscle must depend largely upon progressive thickening of these bundles as the animal grows older and larger.

2. Number of fibres comprising the bundle.

For counting the number of muscle fibres comprising the individual bundle, cross-sections were prepared by cutting frozen sections at the centre of the muscle (site C). The fibres of twenty bundles, selected at random, were counted from each muscle. The results are shown in Table 26.

TABLE 26.
Number of muscle fibres per bundle.

GROUPS OF RABBITS.		Number of Rabbits.	Fibres. (<i>M. Gastroc.</i>)	Significance Test (<i>W. Group 1</i>)	Fibres (<i>M. Psoas</i>).	Significance Test (<i>W. Group 1</i>).
No.	Class.					
1	Birth . . .	10	48		92	—
7	600 gm. . .	10	51	N.S.	91	N.S.
8	1,200 gm. . .	10	52	N.S.	87	N.S.
9	1,800 gm. . .	10	60	N.S.	95	N.S.
10	2,400 gm. . .	10	54	N.S.	91	N.S.
11	3,000 gm. . .	10	50	N.S.	94	N.S.
12	Mature . . .	10	51	N.S.	87	N.S.

It is not possible to differentiate the number of fibres per bundle between birth and maturity, both in *M. Gastrocnemius* and in *M. Psoas*. Not one of the variations is significant so that all may be due to chance alone. Thus the differentiation of muscle fibres has ceased at birth, and the post-natal growth of muscle must be attributed to an increase in size of the fibres existing at birth.

When the two muscles are compared, it is seen that *M. Gastrocnemius* has consistently less fibres per bundle than *M. Psoas*. Nevertheless, *Gastrocnemius* bundles will not necessarily be more slender than those of the *Psoas* muscle, as differences in fibre diameter must also be taken into account. This aspect is discussed in the following section.

3. Cross-sectional area of muscle bundle ("grain", texture).

This has been calculated from the number of fibres per bundle, together with the mean fibre diameter for each group (Table 27, Figure 17). If the fibre is presumed to be circular in outline, the mean cross-sectional area can be calculated from the mean fibre diameter. This, multiplied by the average number of fibres constituting the bundle, gives the average cross-sectional area of the muscle bundle.

In both muscles "grain" increases more or less uniformly from birth to 3,000 gm. live-weight, with only a slight increase from 3,000 gm. to maturity. All increments are significant in *M. Psoas*, except for the mature animal, but in *M. Gastrocnemius* significance is just missed at both 2,400 gm. live-weight and 3,000 gm.

TABLE 27.

Cross-sectional area of muscle bundle.

GROUPS OF RABBITS.		No. of Rabbits.	Mean Area. (M. Gastroc.).	Significance Test. (W. Prec. Group).	Mean Area. (M. Psoas.)	Significance Test. (W. Prec. Group).
No.	Class.					
			Sq. Mm.		Sq. Mm.	
1.....	Birth..	10	.004,880	—	.005,212	—
7.....	600 gm..	10	.052,877	XX	.027,339	XX
8.....	1,200 gm..	10	.090,983	XX	.057,560	XX
9.....	1,800 gm..	10	.181,098	XX	.108,428	XX
10.....	2,400 gm..	10	.199,320	N.S.*	.133,238	XX
11.....	3,000 gm..	10	.221,077	N.S.*	.152,538	X
12.....	Mature....	10	.246,405	X	.153,952	N.S.

* Here significance at P — 0.05 is just missed.

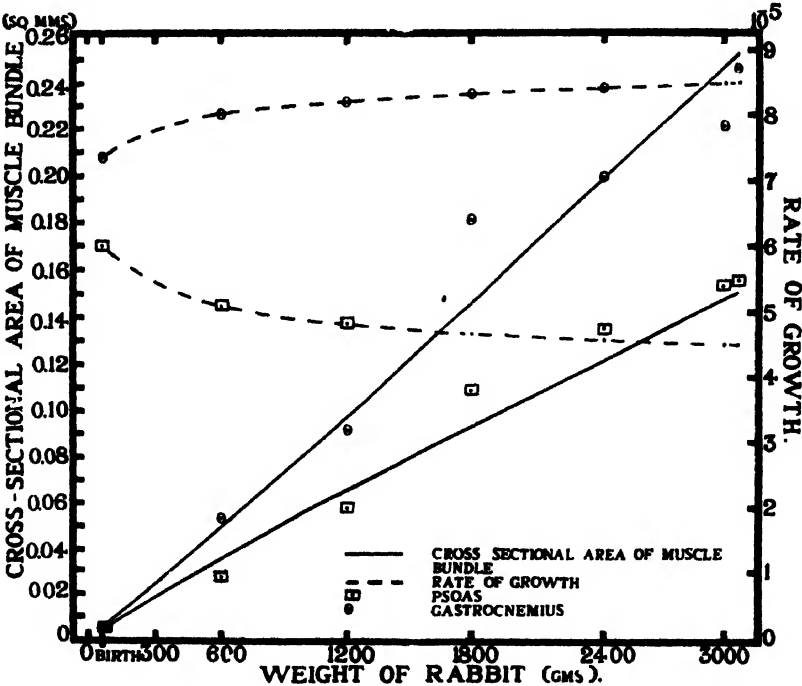


Fig 17.- Cross-sectional area of muscle bundle.

Comparison of the two muscles shows that at birth M. Psoas has slightly bigger bundles, but they become increasingly smaller than those of M. Gastrocnemius in the succeeding stages from 600 gm. live-weight to maturity. Thus, in general, M. Psoas is a finer grained muscle, this difference becoming accentuated as the animal grows older.

The reason for this is evident from a study of rate of growth (Table 28, Figure 17). *M. Gastrocnemius* has always a greater growth rate than *M. Psoas*. *Gastrocnemius* bundles show a pronounced increase between birth and 600 gm. live-weight, after which the rate slackens to become almost stationary from 2,400 gm. onwards. On the other hand, *M. Psoas* is growing fastest at birth and the growth rate then drops markedly to 600 gm. live-weight. From 600 gm. the curve declines more gradually to become flat in the ultimate stages.

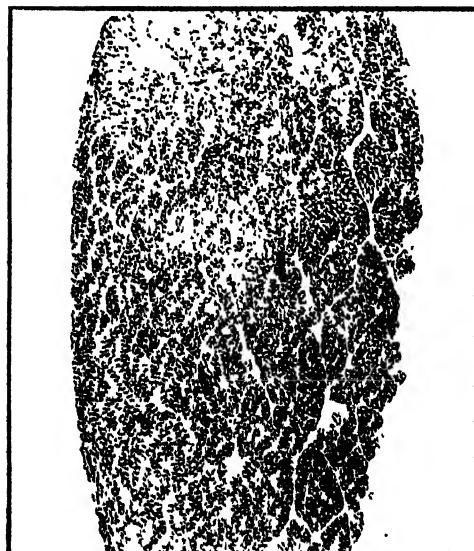
TABLE 28.
Rate of growth of cross-sectional area of muscle bundle.

Group.	<i>M. Gastrocnemius.</i>	<i>M. Psoas.</i>
Birth.....	·000,074	·000,060
600 gm.....	·000,080	·000,051
1,200 gm.....	·000,082	·000,048
1,800 gm.....	·000,083	·000,047
2,400 gm.....	·000,084	·000,046
3,000 gm.....	·000,085	·000,045
Mature.....	·000,085	·000,045

PLATE III.

The effect of age on the coarseness of texture in the Gastrocnemius muscle.
(All to the same magnification 50×.)

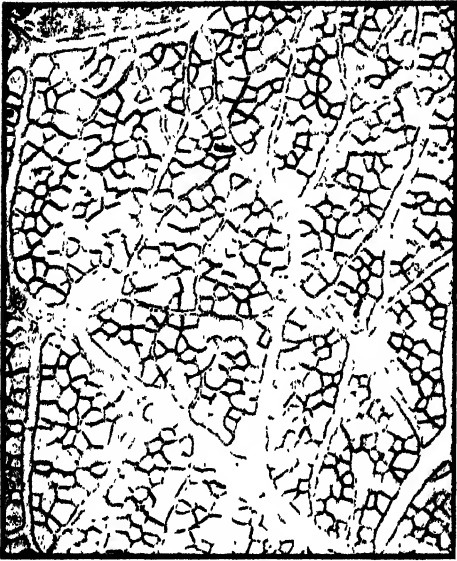
Unstained cross-sections cut by the freezing method. Large bundles, consisting of a smaller number of thicker fibres, than in the *Psoas* muscle.



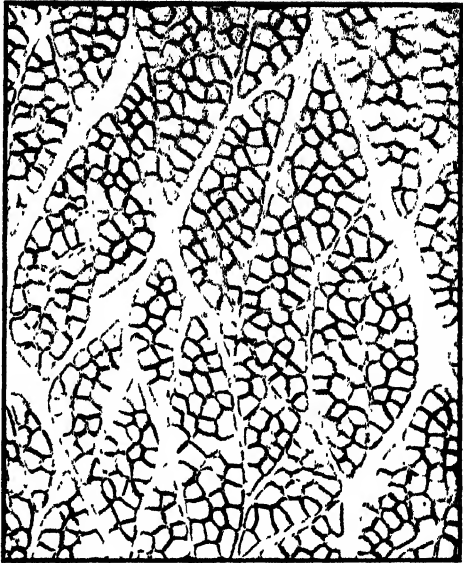
Birth.



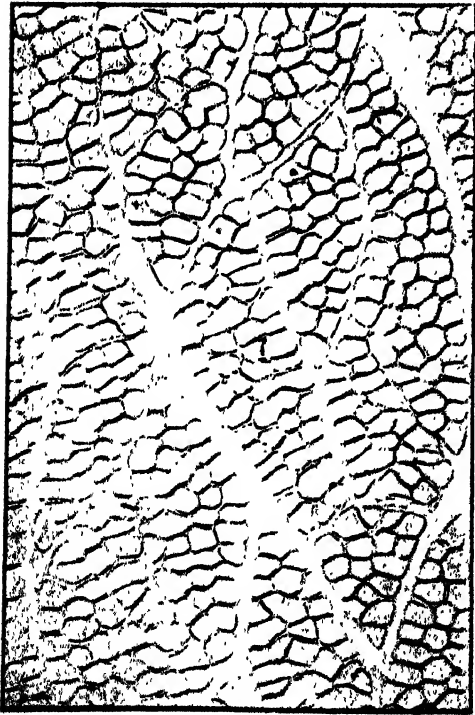
600 gm. live-weight.



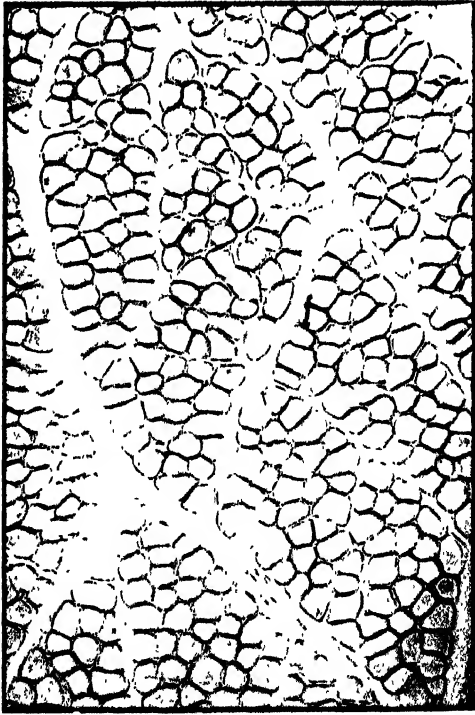
1,200 gm. live-weight.



1,800 gm. live-weight.



2,400 gm. live-weight.



3,000 gm. live-weight.



"Mature."

PLATE IV.

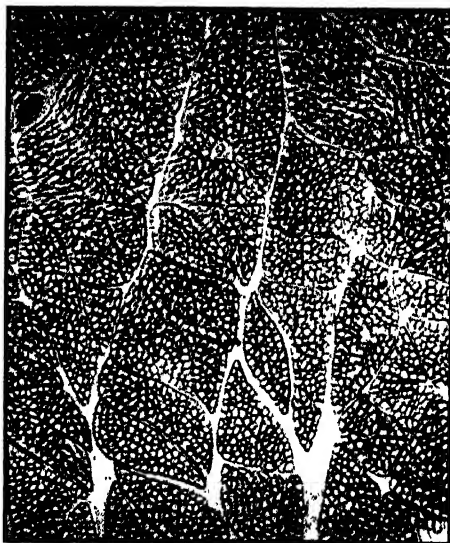
The effect of age on the coarseness of texture in the Psoas muscle.

(All to the same magnification 50 ×.)

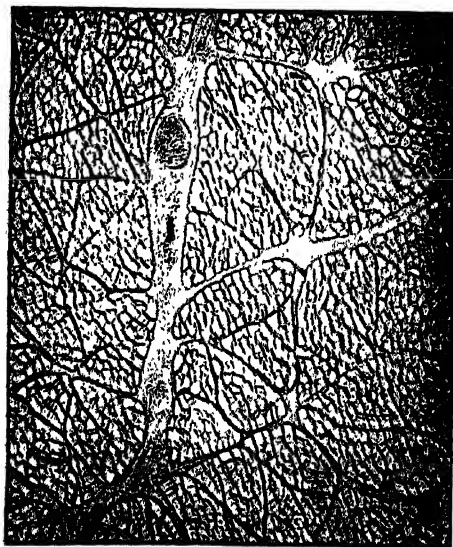
Unstained cross-sections cut by the freezing method. Small bundles, compared with the Gastrocnemius muscle, consisting of a larger number of thinner fibres. Note the marked dissimilarity in size of the component fibres.



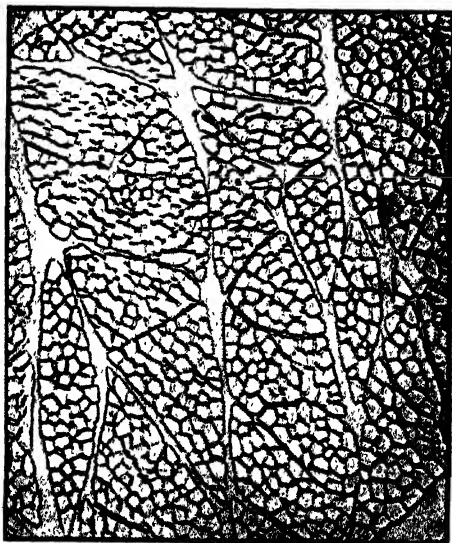
Birth.



600 gm. live-weight.



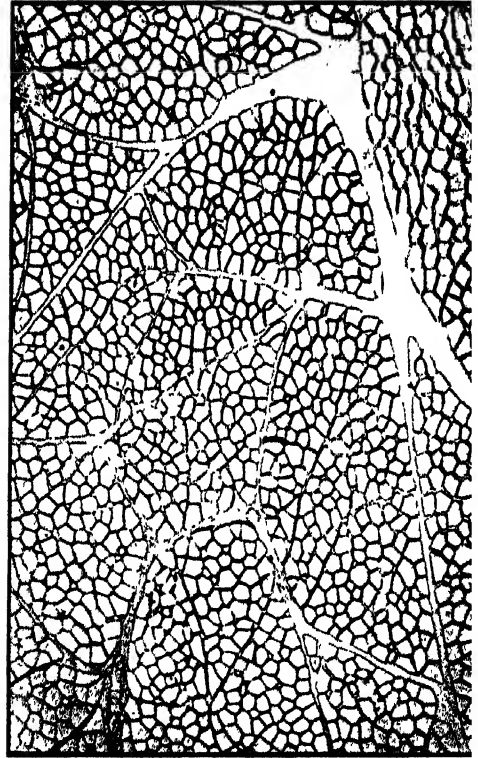
1,200 gm. live-weight.



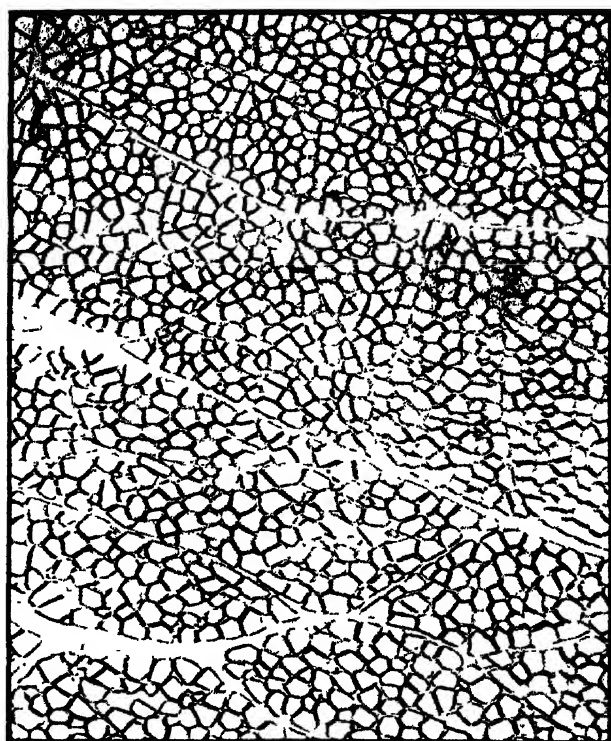
1,800 gm. live-weight.



2,400 gm. live-weight.



3,000 gm. live-weight.



Mature."

Discussion.

In order to emphasize the growth changes, a diagrammatic representation of the muscle bundle as a whole is shown in Figure 18. Obviously, it is difficult to draw to the same scale, length in terms of centimetres and "thickness" (cross-sectional area) in fractions of a square millimetre. In this diagram length has been correctly scaled for both muscles, so that the proportions are true to life. "Thickness" has been drawn to a larger scale. Again, the relative "thickness" is correct for both muscles, but the proportion of length to "thickness", considered as separate characters, is false.

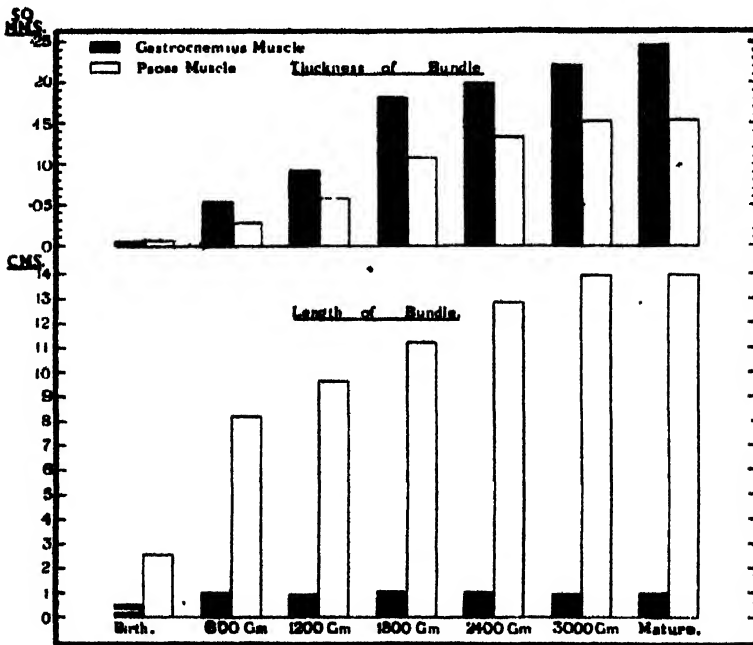


Fig. 18.—Growth of muscle bundle in length and thickness

Length growth of the two types of bundles is clearly dissimilar. Excluding the period from birth to 600 gm. live-weight, there is virtually no change in length of the Gastrocnemius bundle. On the other hand, the much longer Psoas bundles lengthen, in stages spread more or less evenly, over the period up to 3,000 gm. live-weight. Clearly length growth of the Psoas bundle is a much later developing character than in the Gastrocnemius muscle. The degree of thickening of the bundle is more or less the same in both muscles, but the Gastrocnemius bundles are thicker at all stages of the growth period after birth.

Thus the increase in substance of *M. Gastrocnemius*, from 600 gm. live-weight to maturity, may be attributed to a thickening of the component bundles. Lengthening of bundles plays no part in this process. By contrast, the Psoas muscle grows largely by lengthening of the bundles. Although thickness growth of the bundles also contributes to enlargement of the muscle, it is probable that the length increase is the more important factor.

From the viewpoint of meat research, interest is also attached to the eating quality of these different types of muscle. It is to be expected that a finely grained muscle will be less stringy or tough (Hammond 1940a; 1940b), but other factors such as the amount of connective tissue and fat have to be considered. *M. Psoas* (or fillet) is traditionally a delicacy, with probably the most tender meat in the carcass. How far is this to be attributed to fineness of the muscle bundles? The tougher *Gastrocnemius* muscle has been found to contain a large amount of connective tissue, in addition to the coarseness of grain here revealed, but the respective importance of each factor in determining toughness has still to be decided.

Obviously the texture of a muscle is dependent on the number of fibres constituting the bundles, as well as the thickness of these fibres. As both these factors must be such as to promote muscular efficiency, the reasons for the inherent differences, shown in the muscles studied, are to be found by study of the physiological characteristics of varying types of muscle. In general, thicker fibres (and bundles) are to be expected in a muscle used mainly for maintaining posture than in a muscle employed for movement. No doubt the number of fibres constituting the bundle depends on the activity of contraction, as well as the functional strength required during normal muscle contraction. Only by investigation of muscle function will it be possible to explain the basic reason for the variations in architecture of various types of muscle, such as have been shown for *M. Psoas* and *M. Gastrocnemius*.

(d) GROWTH AND DEVELOPMENT OF MUSCLE FIBRE.

(Literature: Pages 341-347.)

A study of the morphology of the growing muscle fibre may throw light on the mechanism of muscle growth and development, which must ultimately depend on changes initiated in the microstructure of the muscle. Hence, it is necessary to pass on to a consideration of the individual muscle fibre. It will be appreciated, however, that the technical difficulties, inherent in measurement of minute units such as the muscle fibre, make it difficult to obtain a complete picture of the morphological changes occurring in the growing fibres.

1. *Length of muscle fibre.*

M. Gastrocnemius.

Evidence has been presented that, in the *Gastrocnemius* muscle, the component fibres pass right along the length of the bundle from one end to the other (pages 341-342). In order to obtain confirmation of this finding it was necessary to carry out considerable work.

After separating individual bundles from a muscle, the bundles were straightened in a holder made for this purpose and securely fastened in this position. Each bundle was infiltrated in an ascending series of gelatin solutions and embedded in twenty-five per cent. gelatin. Serial sections were cut transversely by the freezing method in a low temperature room. All sections were examined microscopically to ascertain the presence of fibres beginning or ending within the bundle. In addition, in every tenth section, the number of fibres comprising the bundle was counted, as well as measuring the diameter of each fibre within the bundle. The results obtained substantiate the claim that *Gastrocnemius* fibres extend from one end of the bundle to the other end.

This is of importance, as an estimate of fibre length can be obtained relatively easily by measuring length of bundle. The observations regarding Gastrocnemius bundles have already been discussed (page 392). For purposes of comparison, length of the Gastrocnemius muscle fibre may be considered to be equivalent to bundle length. Therefore, as in the case of bundle length, length of the Gastrocnemius fibre is an extremely early developing character. By the time that the animal has attained a live-weight of 320 gm. fibres have ceased to make any further length growth. Furthermore, a gradient of fibres of varying lengths exists within the muscle. In general, fibres are shorter at B than at A, and show a progressive increase in length at each of the succeeding sites C, D, and E.

M. Psoas.

Unlike the Gastrocnemius muscle, Psoas fibres are usually shorter than the bundle with one or both ends lying freely within the bundle. This makes it difficult to devise a method for obtaining a routine measurement of length of fibre, to be applied in this experiment. No suitable technique was devised, hence it is not possible to present any data concerning the length growth of Psoas muscle fibres. However, from a consideration of bundle length (page 397), it would appear that the fibres constituting these bundles continue to lengthen over a relatively greater period of the lifetime of the animal, than in the Gastrocnemius muscle.

2. Diameter of Muscle Fibre.

M. Gastrocnemius.

Fibre diameter is considered in Table 29 and Figure 19.

TABLE 29.
Diameter of Gastrocnemius Muscle Fibres.

RABBIT GROUP.		No. of Rabbits.	DIAM.	Sig. Test	DIAM.	Sig. Test	DIAM.	Sig. Test	DIAM.	Sig. Test	DIAM.	Sig. Test
No.	Class.		" A "	Proc. Gr.	" B "	Proc. Gr.	" C "	Proc. Gr.	" D "	Proc. Gr.	" E "	Proc. Gr.
1	Birth...	10	μ 12.30	—	μ 11.33	—	μ 10.83	—	μ 10.91	—	μ 11.41	—
7	600 Gm.	10	33.87	XX	34.74	XX	36.79	XX	39.57	XX	37.12	XX
8	1,200	10	42.23	XX	45.85	XX	48.85	XX	51.18	XX	48.24	XX
9	1,800	10	55.22	XX	58.55	XX	62.30	XX	67.78	XX	66.23	XX
10	2,400	10	59.93	X	65.61	XX	68.06	XX	74.44	XX	74.84	XX
11	3,000	10	64.40	X	72.06	XX	75.48	XX	81.55	XX	81.82	X
12	Mature.	10	67.04	N.S.	75.20	N.S.	77.43	N.S.	85.64	N.S.	87.38	X

Considerable thickening of fibres takes place from birth to maturity. After a period of marked increase from birth to 600 gm. live-weight the curves flatten out progressively in the subsequent groups, so that there is only a slight increase between 3000 gm. and maturity. This flattening of the curves takes place in a graded manner, least and latest at D and E, and in order of increasing flatness C, B, and A. Thickening of fibres near the muscle insertion clearly continues over a longer period of the life of the animal, than at points nearer the muscle origin. Significance is shown for all increments in fibre diameter at each site, except in the mature group of rabbits.

In Table 30 and Figure 19 are to be found details concerning the rate of growth. The growth rate is greatest at birth, then decreases sharply until 600 gm. live-weight, after which the curves flatten less abruptly until 1800 gm., and become nearly horizontal in the concluding groups. It is to be noted the same relative order is preserved throughout, namely D, E, C, B, A, in order of decreasing magnitude.

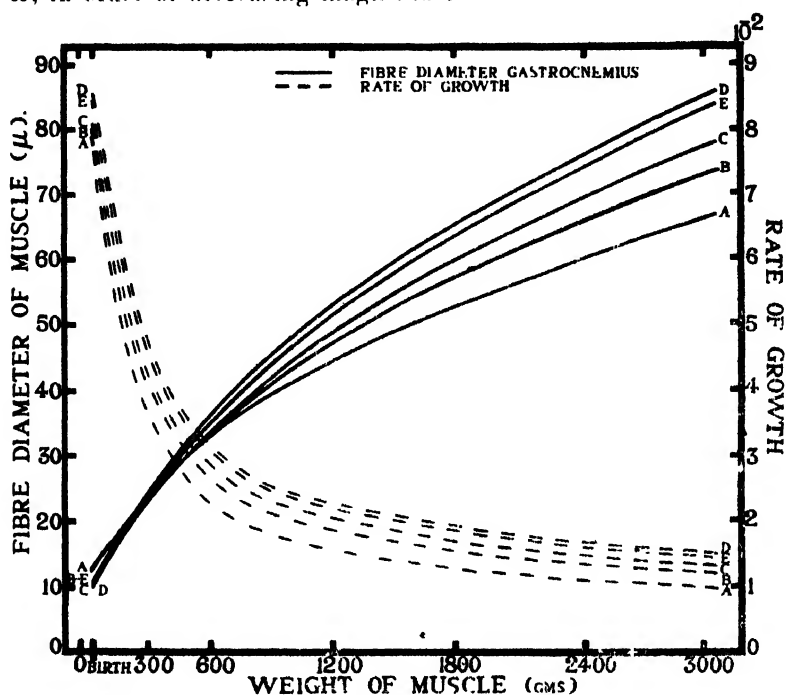


Fig. 19 - Diameter of Gastrocnemius muscle fibres. [Corrigendum For legend-abscissa axis substitute WEIGHT OF RABBIT (GMS)]

TABLE 30.

Rate of Growth of Fibre Diameter in M. Gastrocnemius.

Group.	Site A.	Site B.	Site C.	Site D.	Site E.
Birth.....	·077,991	·079,868	·081,428	·085,643	·085,240
600 gm.....	·023,095	·026,433	·028,272	·031,124	·030,491
1,200 gm.....	·015,990	·018,919	·020,535	·022,916	·022,341
1,800 gm.....	·012,861	·015,520	·016,990	·019,113	·018,582
2,400 gm.....	·010,918	·013,371	·014,732	·016,676	·016,176
3,000 gm.....	·009,720	·012,031	·013,317	·015,141	·014,663
Mature.....	·009,611	·011,907	·013,186	·014,095	·014,522

Relative fibre diameter within the muscle is considered in Table 31 and Figure 20. The most striking feature of the table is that at birth relative fibre diameter presents a pattern different from that of the subsequent groups.

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TABLE 31.
Relative Fibre Diameter of Gastrocnemius Muscle.

Site in Muscle.	BIRTH.			900 Gm.			1,200 Gm.			1,800 Gm.			2,400 Gm.			3,000 Gm.			MATURE.		
	Diam.	Sig. Test.		Diam.	Sig. Test.		Diam.	Sig. Test.		Diam.	Sig. Test.		Diam.	Sig. Test.		Diam.	Sig. Test.		Diam.	Sig. Test.	
		Prec.	Site		Prec.	Site		Prec.	Site		Prec.	Site		Prec.	Site		Prec.	Site		Prec.	Site
	μ			μ			μ			μ			μ			μ			μ		
A.....	12-30	—	—	33-87	—	—	42-23	—	—	55-22	—	—	59-93	—	—	64-40	—	—	67-04	—	—
B.....	11-33	XX	XX	34-74	N.S.	N.S.	45-35	X	X	58-55	X	X	65-61	XX	XX	72-06	XX	XX	75-29	XX	XX
C.....	10-33	N.S.	XX	36-79	XX	XX	48-85	XX	XX	62-30	XX	XX	68-06	X	XX	75-48	XX	XX	77-43	N.S.	XX
D.....	10-91	N.S.	XX	39-57	XX	XX	51-18	N.S.	XX	67-78	XX	XX	74-44	XX	XX	81-55	XX	XX	85-04	XX	XX
E.....	11-41	N.S.	XX	37-12	XX	XX	48-24	X	XX	66-23	N.S.	XX	74-94	N.S.	XX	81-82	XX	XX	87-38	N.S.	XX

ABBREVIATIONS: Sig. Test. Significance Test.

TABLE 32.
Relative fibre diameter of Psoas muscle.

Site in Muscle.	BIRTH.			600 Gm.			1,200 Gm.			1,800 Gm.			2,400 Gm.			3,000 Gm.			MATURE.		
	Diam.	Sig. Test.		Diam.	Sig. Test.		Diam.	Sig. Test.		Diam.	Sig. Test.		Diam.	Sig. Test.		Diam.	Sig. Test.		Diam.	Sig. Test.	
		Prec.	Site		Prec.	Site		Prec.	Site		Prec.	Site		Prec.	Site		Prec.	Site		Prec.	Site
	μ			μ			μ			μ			μ			μ			μ		
A.....	8-74	—	—	20-28	—	—	31-42	—	—	39-50	—	—	44-99	—	—	46-90	—	—	48-68	—	—
B.....	8-39	N.S.	N.S.	19-40	X	X	28-67	XX	XX	38-19	N.S.	N.S.	42-45	XX	XX	43-58	XX	XX	46-21	X	X
C.....	8-38	N.S.	N.S.	19-34	N.S.	X	28-30	N.S.	XX	36-70	N.S.	XX	41-57	N.S.	XX	43-53	N.S.	XX	45-96	N.S.	XX
D.....	8-32	N.S.	X	19-16	XX	XX	27-09	N.S.	XX	37-04	N.S.	XX	42-25	N.S.	XX	44-76	N.S.	X	46-12	N.S.	X
E.....	8-67	N.S.	N.S.	19-37	N.S.	X	29-03	X	XX	38-55	N.S.	N.S.	44-52	X	N.S.	47-56	XX	N.S.	49-95	XX	N.S.

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At birth, fibre diameter is greatest near the origin of the muscle (A), and smaller at the subsequent points (B, C, D, E). The significance test shows a positive result throughout. In the later groups, from 600 gm. live-weight to maturity, the thinnest fibres are found near the origin (A), with a significant increase in diameter at the succeeding points B, C, and D. One negative result is shown in both the 600 gm. and the 1200 gm. groups. Excluding birth, the fibres at E are in some groups thinner than at D (600 gm., 1200 gm.), whereas no significant difference is shown for the other groups (1800 gm., 2400 gm., 3000 gm., maturity).

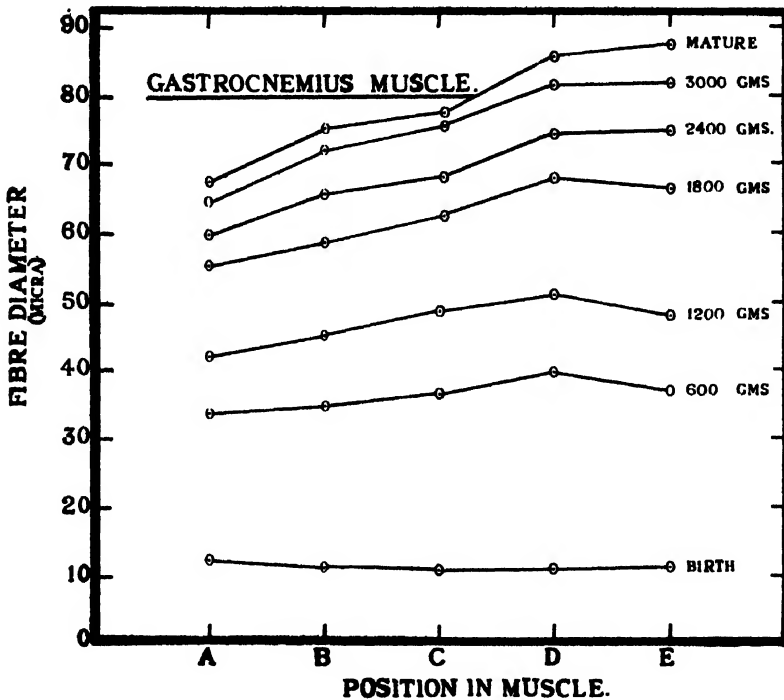


Fig. 20. Relative fibre diameter of Gastrocnemius muscle.

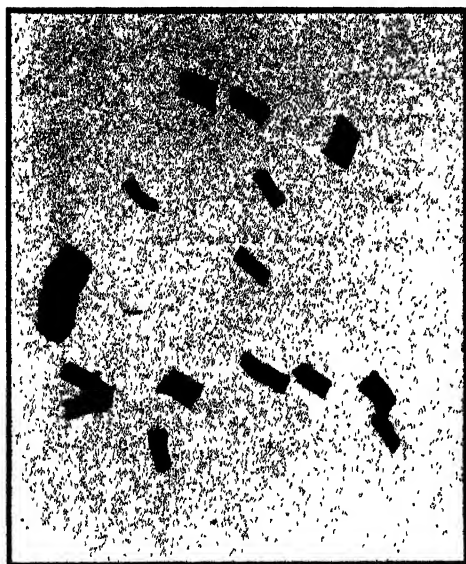
This latter irregularity may be due to the variation in individual animals comprising the groups (see Appendix Table F). Fibres at the terminal site E are thinner than at D in 8, 6, 7, 6, 6 and 2 animals respectively of the ten rabbits comprising each of the last six groups. Perhaps slight differences in the relative positions at which fibres were taken for measurement from different animals may account for this variation. Under the circumstances, all that can be said is that, in the older animals, there is a tendency for the diameter of fibre to become slightly reduced from D towards the insertion of the muscle.

With reference to the change in relative thickness of the fibres in the older groups as compared with the new-born rabbit, probably this is brought about by the growth gradient shown to exist along the length of the muscle. It has been observed that the rate of thickening of fibres increases in progressive manner from the beginning towards the end of the muscle.

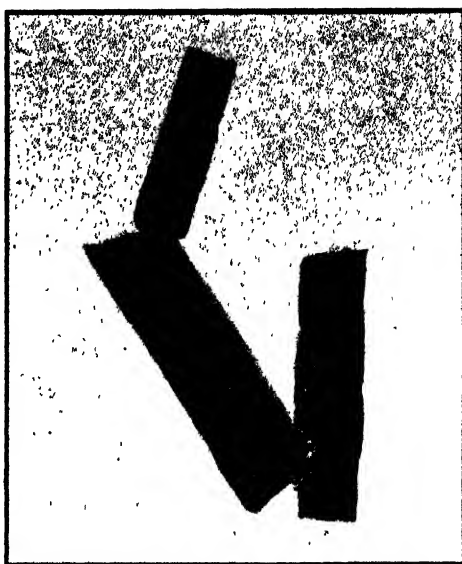
PLATE V.

*The effect of age on the diameter of the muscle fibres of M. Gastrocnemius.
(All to the same magnification 200×.)*

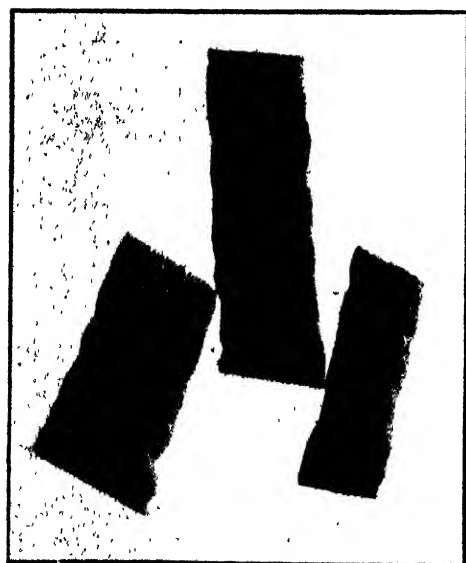
Unstained fibres teased from the formalin-fixed muscle showing striation in both the longitudinal and transverse direction.



Birth.



600 gm. live-weight.



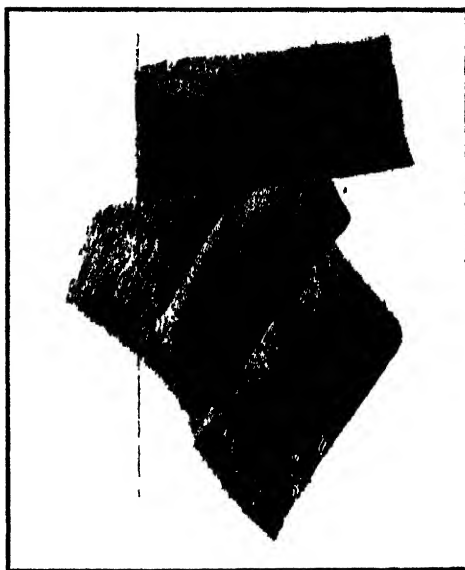
1,200 gm. live-weight.



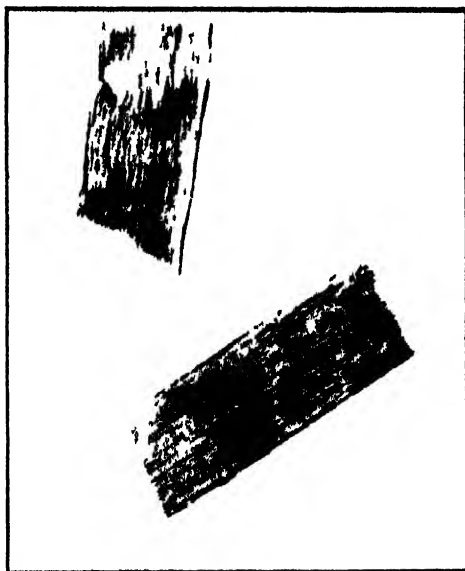
1,800 gm. live-weight.



2,400 gm live-weight



3,000 gm live-weight



" Mature "

M. Psoas.

Throughout the life of the animal, Psoas fibres are appreciably thinner than the Gastrocnemius fibres. Although there is a definite increase in fibre diameter, this increase is much less marked than for *M. Gastrocnemius* (Table 33, Figure 21). The curves are uniformly flatter throughout. From the tests it is seen that there are significant differences at all the stages up to 2400 gm. live-weight. In the two final groups some of the increments do not show significance, hence they may be chance variations. The tendency is for increases in fibre diameter to be less definite from 2400 gm. live-weight onwards than in the *Gastrocnemius* muscle.

TABLE 33.

Diameter of Psoas Muscle Fibres.

Rabbit Group.		No. of Rabbits.	DIAM.	Sig. Test	DIAM.	Sig. Test	DIAM.	Sig. Test	DIAM.	Sig. Test	DIAM.	Sig. Test
No.	Class.		"A"	Proc. Gr.	"B"	Proc. Gr.	"C"	Proc. Gr.	"D"	Proc. Gr.	"E"	Proc. Gr.
1	Birth...	10	μ 8.74	—	μ 8.39	—	μ 8.38	—	μ 8.32	—	μ 8.67	—
7	600 Gm.	10	20.28	XX	19.40	XX	19.34	XX	19.16	XX	19.37	XX
8	1,200	10	31.42	XX	28.67	XX	28.30	XX	27.69	XX	29.03	XX
9	1,800	10	39.50	XX	38.19	XX	36.70	XX	37.04	XX	38.55	XX
10	2,400	10	44.99	XX	42.45	XX	41.57	XX	42.25	XX	44.52	XX
11	3,000	10	46.90	N.S.	43.58	N.S.	43.53	N.S.	44.76	X	47.56	X
12	Mature.	10	48.68	N.S.	46.21	X	45.96	X	46.12	N.S.	49.95	X

Rate of growth is considered in Table 34 and Figure 21. At birth, the Psoas growth rate is roughly two-thirds of the rate for *M. Gastrocnemius*. However, from 600 gm. live-weight to maturity it is only slightly less than the *Gastrocnemius* growth rate. From the graphs it is seen that the curves are of similar trend in both muscles. After an initial steep decline to the 600 gm. group, the rate of growth subsequently flattens out gradually to become almost horizontal in the final stages. There is a tendency for a gradient to occur along the length of the muscle, whereby the growth rate at the ends (A, E) is higher throughout than in the central portion (B, C, D). These differences are less marked than those encountered in the *Gastrocnemius* muscle.

TABLE 34.

Rate of growth of fibre diameter in M. Psoas.

Group.	Site A.	Site B.	Site C.	Site D.	Site E.
Birth.....	.054,717	.051,898	.051,380	.051,314	.053,530
600 gm.....	.016,861	.015,824	.015,537	.015,754	.016,713
1,200 gm.....	.011,815	.011,052	.010,824	.011,024	.011,757
1,800 gm.....	.009,570	.008,934	.008,738	.008,923	.009,546
2,400 g.....	.008,166	.007,614	.007,438	.007,612	.008,161
3,000 gm.....	.007,299	.006,798	.006,636	.006,801	.007,303
Mature.....	.007,219	.006,721	.006,562	.006,726	.007,223

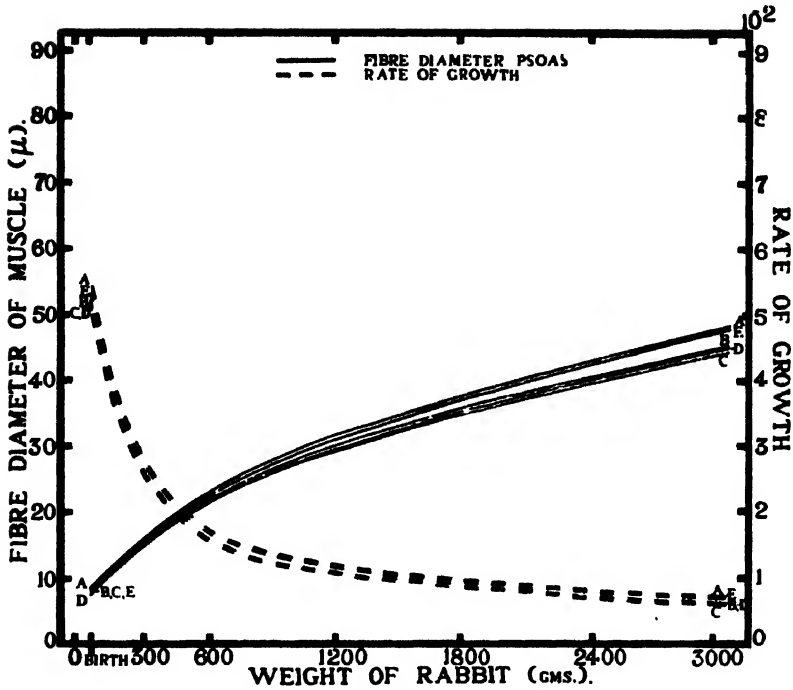


Fig. 21.—Diameter of Psoas muscle fibres.

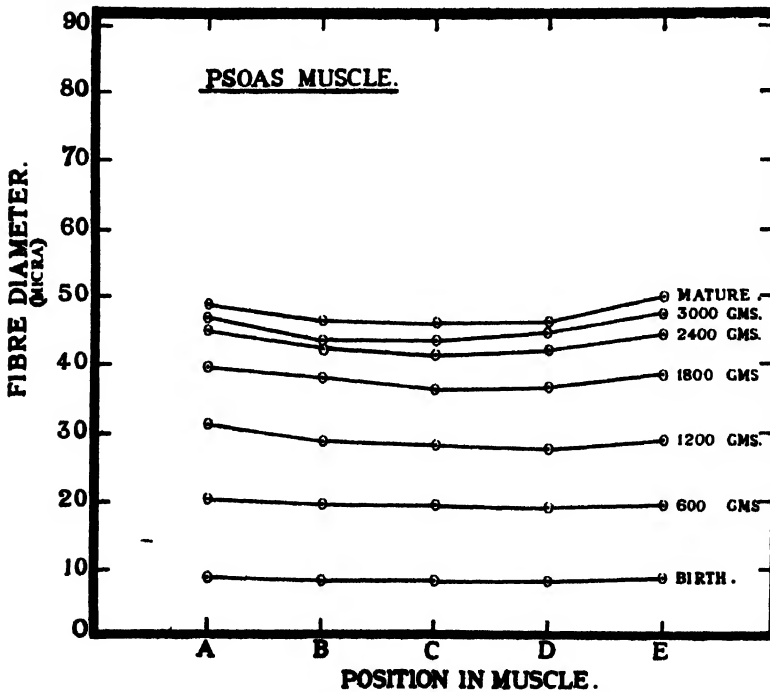


Fig. 22.—Relative fibre diameter of Psoas muscle.

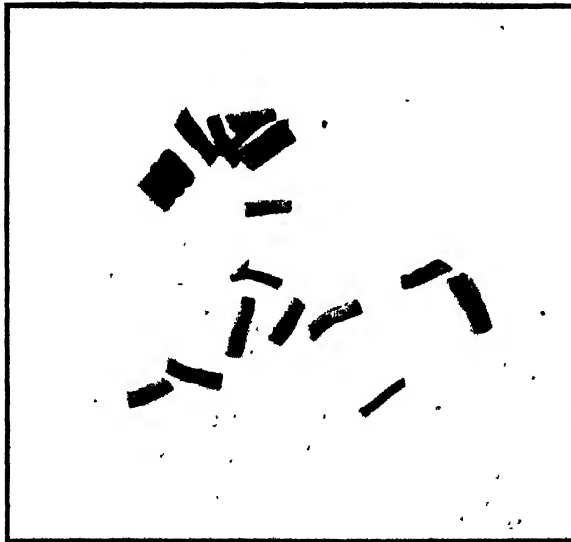
Details of relative fibre diameter within the muscle are presented in Table 32 and Figure 22. The fibres tend to become progressively thinner from A to B to C, but they tend to thicken again towards the other end of the muscle, from C to D to E. In general, fibre diameter at B, C, and D, is significantly less than at site A (two exceptions at birth, one exception at 1,800 gm. live-weight). Only a slight difference is evident between fibre diameter at the two ends of the muscle, as the fibres are significantly thinner at E than at A only in the 600 gm. and 1,200 gm. groups. In all the other groups the variations may be due to chance alone. By comparison with the picture painted for *M. Gastrocnemius*, thicker fibres are found at the ends of the *Psoas* muscle, and the central portion of the muscle is composed of thinner fibres.

PLATE VI.

The effect of age on the diameter of the muscle fibres of M. Psoas.

(All to the same magnification $200\times$.)

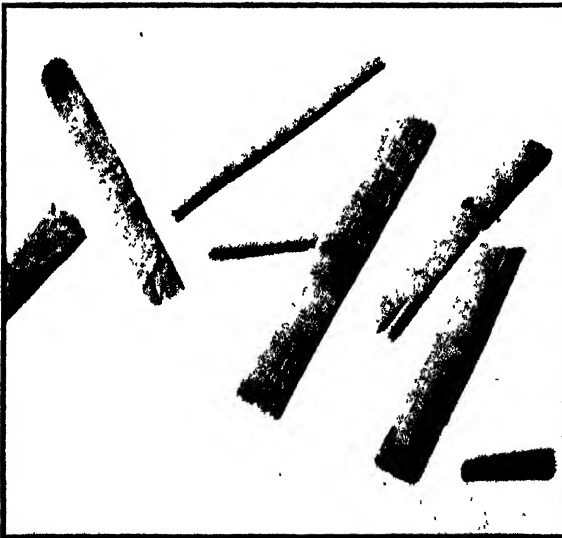
Unstained fibres, teased from the formalin-fixed muscle, showing a characteristic broad cross-striation compared with *Gastrocnemius* fibres. Note the marked variation in size, here illustrated particularly for the 1,200 gm. group.



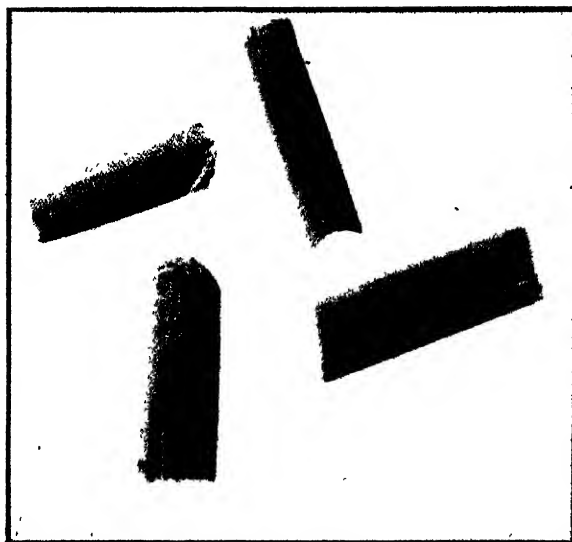
Birth.



600 gm. live-weight.



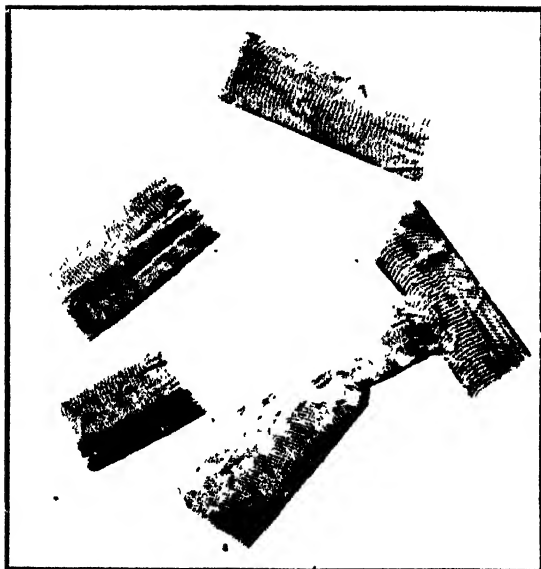
1,200 gm. live-weight.



1,800 gm. live-weight.



2,400 gm. live-weight.



3,000 gm live-weight.



"Mature."

Discussion.

M. Gastrocnemius. Gastrocnemius fibres attain their maximum development in length very early in life (320 gm. live-weight—52 days). However, the thickness of these fibres continues to increase throughout the lifetime of the animals studied, although a definite increase is not shown between 3000 gm. live-weight and maturity (or over the last six months of the lifetime of these rabbits). Thus, thickening of the fibres must account largely for the muscular development which takes place. Even though *M. Gastrocnemius* is a relatively early developing unit, its component fibres achieve maturity in thickness growth at a late stage of its development. A diagrammatic representation of the respective changes is shown in Figure 23.

It is understandable that the relative thickness of fibres within the muscle at birth presents a pattern different from that of succeeding groups. Rabbits are born in a comparatively immature condition, with relatively little work for the leg muscles early in life. However, when the animals start walking these muscles are in constant use. Not only does this increased functional activity necessitate thicker fibres, but presumably the nature of this work is such as to also call for the development of stronger and thicker fibres towards the insertion of the muscle, in order to promote efficiency of function. A reason cannot be advanced for this graded thickening of fibres. Information is lacking as regards the part played in the functioning muscle, by the fibres of varying degrees of thickness at different points within the muscle.

From the point of view of obtaining a representative measurement of fibre thickness, it appears that a specimen taken from the middle of the muscle will closely approximate the mean fibre diameter of the whole muscle. Hence, in comparative muscle studies, the labour involved in measuring fibres can be reduced considerably where only a general idea of thickness of fibre is required.

M. Psoas.—Comparison of the number of fibres comprising the muscle bundle shows more or less the same number at later stages of life as at birth. In other words, new fibres are not formed after birth. In the absence of a formation of new fibres, it is reasonable to assume that lengthening of the bundle is brought about by an increase in the length of the existing muscle fibres. Although there is a possibility that the different types of fibre (spindleshaped intrafascicular fibres, blunt tendon-end fibres with one intrafascicular termination) may not contribute evenly to this process, nevertheless it remains true that length increase of fibre, considered as a general measure, will be approximately equal to length increase of bundle. For instance, Buchthal and Lindhard (1939) found that, on the average, fibres shorter than the bundle are more than half the length of the bundle. For convenience, fibre length has been pictured as half the observed length of *Psoas* bundles in the diagram shown in Figure 23.

By analogy from a study of the development of bundles, *Psoas* fibres probably lengthen progressively over the lifetime of the rabbit until 3000 gm. live-weight. This appears to be the major difference between the development of the two muscles. Thickness of fibre does not show as marked development as does length of bundle (or fibre), and the curve is flatter throughout the period studied. As thickening of the fibres is relatively

slight, it is likely the late development of M. Psoas can be attributed mainly to a lengthening of the muscle fibres, continued until a much later stage of life as contrasted with the Gastrocnemius muscle.

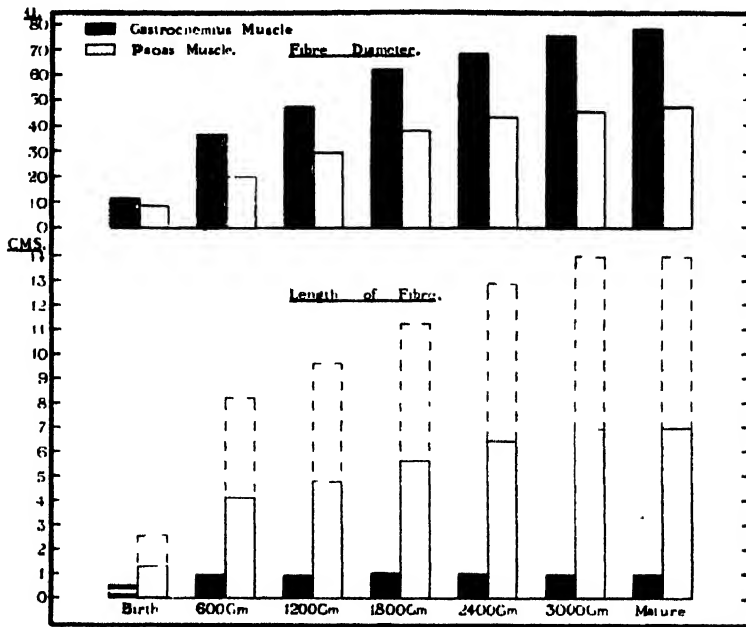


Fig. 23 - Growth of muscle fibre in length and thickness

A factor which may contribute to a thicker measurement of fibres towards the end of the muscle, than in mid-muscle, may be the presence of innumerable fibre ends within the muscle. No idea can be presented of the relative proportion of ends to fibres at the various sites studied but it appears reasonable that there will be more intralascular endings in the vicinity of the mid-muscle. Hence, measurement of these endings of fibres will tend to reduce the mean fibre diameter about this locality. Owing to the great variability in thickness of the Psoas fibres it is not possible during measurement to overlook fine fibres, on the assumption that they represent fibre ends. However, the constant relationship shown to exist in the many individual Psoas muscles studied at different stages of life, seems to indicate a relatively uniform distribution of fibre endings in these different muscles. A specimen taken from the middle of the muscle is likely to give a representative figure, which is slightly less than the mean diameter considered over the whole of the muscle.

Consideration of the muscle function i.e. motile as opposed to postural, appears to account for the relative fineness of Psoas fibres as compared with Gastrocnemius fibres. When the Psoas muscle is called upon to function, relatively slight and quick movement of short duration may be presumed. A large number of small fibres facilitates the task of respiratory exchange in this type of contraction, whereas in M. Gastrocnemius more constantly in use for postural purposes there is possibly an hypertrophy of the fibres, together with the development of an increased amount of myoglobin to meet the respiratory demands.

CHAPTER V.—SUMMARY.

Attention is directed to the paucity of information regarding the morphological growth and development of muscle, particularly in connection with the microscopical elements comprising muscle. Such knowledge is of value, not only in affording a basis for studying meat quality in different species of domestic animals, but also in understanding the principles underlying the function of different muscles.

In a preliminary study, statistical methods were employed in order to ascertain suitability of sampling and measuring muscle bundles and muscle fibres. For sampling the bundle length of a muscle, a selection of ten measurements gives a sufficiently reliable mean. For sampling the diameter of muscle fibres, a selection of one hundred measurements affords a sufficiently reliable mean. Variable results are obtained when a measure of fibre diameter is calculated from cross-sections of muscle, probably due to the difficulty of cutting sections at right angles to the line of the fibres. Measurement of the cross-diameter of short lengths of muscle fibres yields more reliable results, and the values obtained are greater than those obtained by measuring fibre diameter in cross-sections. For calculating the texture of a muscle, a selection of twenty bundles provides a fair estimate of the number of fibres comprising the individual muscle bundle.

With the object of establishing the general principles of morphological development during post-natal life, the relative changes were studied in the tissues and anatomical units of *M. Gastrocnemius medialis* and *M. Psoas major*, in a series of male rabbits killed at intervals from birth to fourteen months of age. Throughout this study, the quantitative data were subjected to statistical analysis. Qualitative changes were not considered. The work is to be regarded as a preliminary investigation, with the purpose of drawing attention to the main principles involved in the growth of the muscles studied.

Both muscles undergo extensive enlargement during the growth of the rabbit. Relative to the *Psoas* muscle, *M. Gastrocnemius* makes a greater proportion of its growth in mass early in life.

Although no important differences were revealed in the mechanism of lengthening of these muscles, there is a striking difference in the manner whereby the individual muscle bundles contribute to this length increase. Whereas the *Psoas* muscle lengthens by virtue of a persistent increase in the length of its component bundles, *Gastrocnemius* bundles do not lengthen after the first two months of life. During the remainder of the lifetime of the rabbit, they do not contribute to the appreciable degree of lengthening which is still manifested by the *Gastrocnemius* muscle. Because of the oblique position of the bundles, thickening of these bundles appears to be the principal factor promoting this increase in length of the muscle. A change in the relative position of these bundles within the *Gastrocnemius* muscle tends to increase the depth of the muscle.

Both muscles vary appreciably in form. *M. Gastrocnemius* is short, with a pronounced belly, and is more or less uniformly deep. *M. Psoas* is long, with a less marked belly formation, and becomes progressively thicker along its length from origin to insertion. In the absence of information regarding the working of these muscles, the advantages of the respective

variations in form in promoting muscular efficiency cannot be discussed. Contrary to expectation, the muscles do not widen or deepen during a later stage of the lifetime of the animal than they increase in length.

Within *M. Gastrocnemius*, the individual bundles show well-defined differential length relationships. In the young rabbit, the bundles show a progressive increase in length along the length of the muscle from its origin to its insertion. In the older rabbit, the bundles are shorter a little distance beyond the muscle origin than at the origin itself, then exhibit a similar increase in length along the muscle to its insertion. The change from one system to the other occurs between 320–480 gm. live-weight, at about 3–4 weeks of age.

In both muscles the bundles continue to thicken throughout the period of growth. Although there is only a slight difference in bundle thickness in the new-born rabbit, *Gastrocnemius* bundles subsequently thicken at a greater rate and become increasingly thicker than the *Psoas* bundles, as the animal becomes older. Hence *M. Gastrocnemius* assumes a coarse texture relative to the *Psoas* muscle.

The bundles comprising the *Psoas* muscle contain a larger number of individual fibres than the *Gastrocnemius* bundles. However, as regards muscle texture, this numerical superiority is more than offset by the greater fineness of the fibre in the *Psoas* bundles.

No evidence was obtained of any decrease in the number of muscle fibres during the post-natal life. Hence, the enlargement in form and the increasing weight of the muscles must be considered to be due mainly to an increase in the size of the existing muscle fibres.

Length of *Gastrocnemius* muscle fibre is an extremely early developing character. In the *Psoas* muscle the fibres continue to lengthen throughout the period of growth. This increase in the length of *Psoas* fibres is largely responsible for the increase in bulk of the muscle.

Within each muscle, the fibres show a well-defined thickness relationship. In *M. Gastrocnemius*, the fibres at birth are thinner in mid-muscle than at both ends of the muscle. At all succeeding stages the *Gastrocnemius* fibres are thinnest near the origin, then become progressively thicker along the muscle to a point near the muscle insertion. By contrast, the *Psoas* fibres are at all stages thinnest in the middle of the muscle. Presumably these differences in the relative size of the contractile units are dictated by functional considerations.

The *Gastrocnemius* fibres thicken to a greater degree and become much thicker than the fibres in *M. Psoas*. Because of the early cessation of length growth in the *Gastrocnemius* muscle fibre, this thickening of the component fibres largely accounts for the increase in the bulk of *M. Gastrocnemius*.

Any application of the data to a different species of animal, or to different muscles, will naturally require caution. However, the general principles for the two basically different classes of muscle studied are probably similar in allied types of a wide variety of muscle. The essential structural difference of these two types is the direction of the muscle fibres. In *M. Gastrocnemius*, of pinnate structure, the short muscle fibres join the tendon at an acute angle, whereas in the *Psoas* muscle, the fibres are

characterised by their parallel arrangement from end to end of the muscle. For a large bulk of muscle, the general conclusions may be of value in providing a basis for further study of muscle belonging to either of these types.

No mention has been made whether the data collected for the various measurements obey Huxley's allometric law. This aspect of the work is undergoing investigation, and will be presented in a future publication. At this stage, it can be stated that a straight-line relationship in logs. can be fitted to all the data by the method of least squares (i.e. allometric growth), excepting Gastrocnemius weight, Gastrocnemius depth, and muscle length and bundle length for both muscles. However, graphical analysis by means of confidence-regions eliminates in addition a number of the measurements showing a straight-line relationship in logs. by the least squares method. Thus, the method of curve-fitting *completely* satisfying the confidence region criterion shows that M. Gastrocnemius grows in allometric manner only for fibre diameter. Similar treatment for the Psoas muscle leaves only weight, width, and depth of muscle in the category of measurements which obey Huxley's law. Until it is possible to elaborate the reasons for the discrepancies, as well as the dissimilarities in the muscles studied, no useful purpose can be served by discussion or comparison.

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TABLE F.
Details of muscle bundles and muscle fibres.

Serial No. of Rabbit.	M. GASTROCNEMIUS MEDIALIS.										Area of Bundle in Cross-section (Sq. mm.).	
	Ia.	Lb.	Lc.	Ld.	Le.	No. of Fibres in Bundle.	Fa.	Fb.	Fc.	Fd.		Fe.
Birth Group.												
93.....	—	—	—	—	—	57	13.57	11.85	12.41	11.54	12.11	0.005,703,5
94.....	—	—	—	—	—	43	11.25	10.96	10.74	10.77	10.67	0.005,299,4
95.....	—	—	—	—	—	49	10.60	10.24	10.88	10.22	9.78	0.003,610,8
96.....	—	—	—	—	—	43	12.37	10.99	10.00	10.38	11.67	0.004,724,6
97.....	—	—	—	—	—	45	12.03	11.09	9.89	12.21	13.30	0.004,623,1
98.....	—	—	—	—	—	46	12.42	10.52	9.38	9.77	11.02	0.003,986,1
100.....	—	—	—	—	—	49	11.65	10.82	9.95	10.06	11.73	0.004,245,3
101.....	—	—	—	—	—	46	13.39	12.51	12.68	12.20	12.16	0.006,100,1
102.....	—	—	—	—	—	53	12.72	11.61	10.96	10.95	10.55	0.004,662,4
103.....	—	—	—	—	—	48	13.03	12.67	11.44	11.00	11.09	0.005,845,3
Mean	—	—	—	—	—	48	12.30	11.33	10.83	10.91	11.41	0.004,880,1
220 Gm. Group.												
186.....	0.56	0.67	0.73	0.71	0.67	—	—	—	—	—	—	—
188.....	0.60	0.65	0.68	0.66	0.65	—	—	—	—	—	—	—
191.....	0.64	0.65	0.69	0.71	0.73	—	—	—	—	—	—	—
194.....	0.76	0.81	0.82	0.82	0.85	—	—	—	—	—	—	—
197.....	0.83	0.83	0.86	0.88	0.90	—	—	—	—	—	—	—
Mean.....	0.68	0.72	0.75	0.76	0.76	—	—	—	—	—	—	—

Abbreviations as follows:—
 Ia, Lb., etc..... Length of bundle in centimetres at A, at B, etc.
 Fa, Fb, etc..... Fibre Diameter in microns at A, at B, etc.

TABLE F. (continued 2).
Details of muscle bundles and muscle fibres.

Serial No. of Rabbit.	M. GASTROCNEMIUS MEDIALIS.										Area of Bundle in Cross-section (Sq. mm.).
	La.	Lb.	Lc.	Ld.	Le.	No. of Fibres in Bundle.	Fa.	Fb.	Fe.	Fd.	Fe.
320 Gm. Group.											
183.....	0.92	0.96	0.99	1.03	1.03	—	—	—	—	—	—
193.....	0.95	0.94	0.97	1.00	1.00	—	—	—	—	—	—
196.....	0.87	0.84	0.88	0.90	0.93	—	—	—	—	—	—
199.....	0.92	0.92	0.96	0.98	1.02	—	—	—	—	—	—
201.....	0.89	0.91	0.93	0.94	0.98	—	—	—	—	—	—
Mean.....	0.91	0.91	0.95	0.97	0.99	—	—	—	—	—	—
480 Gm. Group.											
192.....	0.94	0.93	0.94	0.97	0.97	—	—	—	—	—	—
196.....	0.97	0.99	1.03	1.05	1.09	—	—	—	—	—	—
200.....	1.03	0.99	0.99	1.02	1.07	—	—	—	—	—	—
202.....	0.91	0.97	0.99	1.00	1.00	—	—	—	—	—	—
203.....	1.05	1.03	1.04	1.06	1.12	—	—	—	—	—	—
Mean.....	0.98	0.98	1.00	1.02	1.05	—	—	—	—	—	—
600 Gm. Group.											
74.....	0.98	0.86	0.91	0.93	0.97	52	36.76	35.82	40.74	42.46	39.68
76.....	0.78	0.79	0.84	0.88	0.95	49	36.20	35.08	37.20	40.10	35.34
77.....	0.81	0.75	0.80	0.91	0.98	56	33.26	33.38	35.62	34.86	35.42
78.....	0.88	0.85	0.88	0.94	0.98	49	35.94	36.60	37.80	39.76	37.58
79.....	0.92	0.85	0.92	1.00	0.98	50	31.24	36.96	37.44	42.28	36.30
80.....	0.80	0.80	0.83	0.84	0.88	51	34.34	36.68	39.78	40.56	38.68
81.....	1.02	1.05	1.06	1.10	1.06	47	32.06	34.82	35.42	38.42	37.20
82.....	1.05	1.08	1.09	1.09	1.13	51	31.48	35.18	37.44	41.52	37.56
83.....	0.91	0.91	0.96	0.99	0.99	50	32.82	30.92	33.68	38.12	35.46
85.....	0.91	0.90	0.94	0.99	0.99	52	31.58	31.98	32.76	37.66	38.00
Mean.....	0.91	0.88	0.92	0.96	0.99	51	33.87	34.74	36.79	39.57	37.12
											0.062,406
											0.062,061
											0.062,380
											0.064,235
											0.065,047
											0.067,870
											0.046,781
											0.063,774
											0.045,932
											0.048,329

Abbreviations as follows:—
 La, Lb, etc..... Length of bundle in centimetres at A, at B, etc.
 Fa, Fb, etc..... Fibre Diameter in microns at A, at B, etc.

TABLE F. (continued 3).
Details of muscle bundles and muscle fibres.

Serial No. of Rabbit.	M. GASTROCNEMIUS MEDIALIS.											Area of Bundle in Cross-section (Sq. mm.).
	La.	Lb.	Lc.	Ld.	Le.	No. of Fibres in Bundle.	Fa.	Fb.	Fc.	Fd.	Fe.	
1,200 Gm. Group.												
62.....	0.93	0.79	0.93	0.93	0.92	46	39.48	42.34	47.72	49.34	43.96	0.071,769
63.....	0.90	0.75	0.82	0.86	0.88	59	41.08	45.92	50.76	52.52	47.04	0.104,376
64.....	0.89	0.77	0.74	0.78	0.81	53	44.02	48.22	46.84	47.62	47.86	0.091,601
65.....	0.91	0.76	0.82	0.88	0.91	53	42.76	50.06	54.18	56.02	52.04	0.108,312
66.....	0.77	0.70	0.80	0.84	0.94	51	44.68	46.82	43.64	48.44	49.40	0.806,983
69.....	0.84	0.87	0.78	0.84	0.95	55	38.46	40.18	47.88	54.88	49.60	0.092,201
87.....	0.88	0.86	0.92	1.02	1.07	51	47.94	49.30	52.62	53.38	51.42	0.103,898
88.....	0.97	0.89	0.83	0.84	0.95	50	34.26	37.76	40.18	45.84	45.90	0.045,338
89.....	0.86	1.10	0.93	1.01	1.07	52	44.38	47.48	52.76	50.96	42.16	0.092,341
90.....	1.12	0.91	1.07	1.11	1.20	48	45.20	45.38	51.94	52.80	53.02	0.093,008
Mean.....	0.91	0.84	0.86	0.91	0.97	52	42.23	45.35	48.85	51.18	48.24	0.090,983
1,800 Gm. Group.												
43.....	0.93	0.86	0.92	0.94	0.94	67	57.08	58.84	61.60	62.64	59.72	0.189,312
44.....	0.92	0.94	0.96	1.06	1.11	60	55.32	57.56	62.88	64.16	66.80	0.177,309
48.....	1.01	1.03	1.08	1.11	1.18	53	58.32	59.32	61.40	70.88	72.64	0.173,229
50.....	1.09	0.93	0.95	1.01	1.11	62	53.48	56.80	64.04	69.16	64.04	0.184,176
51.....	1.05	0.92	0.91	0.95	0.98	64	50.72	55.44	59.96	71.52	70.52	0.190,922
52.....	0.98	0.93	0.99	1.09	1.11	59	53.00	56.88	60.20	67.80	63.52	0.168,390
53.....	0.92	0.87	0.94	1.01	1.05	59	50.68	62.92	68.12	75.60	78.64	0.218,004
54.....	1.02	1.01	1.05	1.14	1.16	58	50.20	55.24	57.80	58.52	58.40	0.143,004
55.....	1.00	0.83	0.91	0.99	1.18	63	59.08	64.16	63.04	67.00	62.04	0.199,265
58.....	0.93	0.98	1.00	1.03	1.04	54	55.28	58.36	62.00	70.52	67.96	0.167,371
Mean.....	0.99	0.93	0.97	1.03	1.09	60	55.22	58.55	62.30	67.78	66.23	0.181,098

Abbreviations as follows:—

La, Lb, etc..... Length of bundle in centimetres at A, at B, etc.
Fa, Fb, etc..... Fibre diameter in microns at A, at B, etc.

TABLE F. (continued 4).
 Details of muscle bundles and muscle fibres.

Serial No. of Rabbit.	M. GASTROCNEMIUS MEDIALIS.										Area of Bundle in Cross-section (Sq. mm.).
	La.	Lb.	Lc.	Ld.	Le.	No. of Fibres in Bundle.	Fa.	Fb.	Fe.	Fd.	Fe.
2,400 Gm. Group.											
38.....	0.78	0.70	0.74	0.82	0.85	47	64.96	67.92	70.56	80.40	78.88
40.....	1.00	1.00	1.01	1.09	1.12	53	63.00	66.12	69.84	78.68	75.32
41.....	1.00	0.94	0.98	1.05	1.13	57	59.04	60.24	66.92	70.32	73.56
42.....	1.06	1.02	1.01	1.02	1.02	50	56.40	60.64	67.12	75.32	74.36
47.....	1.16	1.12	1.07	1.11	1.15	61	57.76	67.76	70.92	74.56	76.44
49.....	0.87	0.88	0.90	1.01	1.07	58	65.64	69.12	66.72	74.04	71.84
60.....	0.86	0.85	0.87	0.91	0.97	53	58.80	65.72	70.96	74.80	79.72
61.....	1.02	0.92	0.95	1.04	1.17	62	58.80	65.96	63.36	70.00	68.68
75.....	1.08	0.90	0.95	1.06	1.14	49	57.96	67.12	70.76	80.20	79.64
86.....	0.94	0.91	0.94	1.07	1.13	50	56.96	61.52	63.40	66.04	70.00
Mean.....	0.98	0.92	0.94	1.02	1.08	54	59.93	65.61	68.06	74.44	74.84
3,000 Gm. Group.											
39.....	0.90	0.82	0.85	0.94	0.94	48	72.40	80.48	82.92	87.32	95.64
59.....	1.07	0.90	0.89	0.91	0.93	48	71.32	78.08	81.16	88.32	92.16
66.....	1.00	0.91	0.90	0.92	0.97	47	60.64	67.44	71.44	77.60	77.36
109.....	1.05	0.87	0.90	0.97	1.00	53	72.76	77.00	82.52	82.72	82.40
113.....	0.96	0.92	1.05	1.08	1.06	53	57.16	61.48	68.32	74.88	76.32
150.....	1.05	0.85	0.97	1.07	1.12	51	54.88	63.08	70.56	77.60	72.20
167.....	1.01	0.86	0.94	1.02	1.04	53	62.88	71.92	74.96	79.64	72.88
170.....	0.98	0.85	0.89	1.01	0.84	50	63.24	76.96	80.56	86.68	86.28
173.....	0.99	0.87	0.98	1.02	1.06	49	61.12	73.84	71.12	80.04	79.44
176.....	0.77	0.74	0.81	0.82	0.81	46	67.56	70.36	71.28	80.72	83.52
Mean.....	0.98	0.86	0.92	0.98	0.98	50	64.40	72.06	75.48	81.55	81.82

Abbreviations as follows:—

La, Lb, etc..... Length of bundle in centimetres at A, at B, etc.
 Fa, Fb, etc..... Fibre diameter in microns at A, at B, etc.

TABLE F. (continued 5).
(Details of muscle bundles and muscle fibres.)

M. GASTROCNEMIUS MEDIALIS.												
Serial No. of Rabbit.	M. GASTROCNEMIUS MEDIALIS.											Area of Bundle in Cross-section (Sq. mm.).
	La.	Lb.	Lc.	Ld.	Le.	No. of Fibres in Bundle.	Fa.	Fb.	Fc.	Fd.	Fe.	
Mature Group.												
108.....	0.78	0.75	0.76	0.78	0.82	45	72.72	83.00	86.68	106.48	107.80	0.294,867
110.....	0.88	0.78	0.80	0.88	0.99	62	63.28	73.24	75.68	87.72	90.00	0.296,107
111.....	1.04	0.98	1.00	1.03	1.06	53	66.68	70.60	72.36	79.28	90.80	0.240,054
158.....	1.02	0.94	0.96	1.03	1.05	44	63.36	70.16	74.04	87.16	81.12	0.195,269
159.....	0.82	0.81	0.86	0.90	0.93	51	76.44	86.76	93.20	93.28	98.12	0.321,284
168.....	1.11	1.05	1.13	1.17	1.21	51	58.08	66.96	67.04	72.60	67.44	0.176,709
171.....	1.21	1.02	1.04	1.13	1.18	57	70.12	79.60	81.36	90.40	93.68	0.308,628
173.....	0.97	0.92	1.00	1.09	1.03	51	63.96	72.44	73.36	79.16	80.44	0.218,573
174.....	0.89	0.79	0.85	0.90	0.96	42	69.04	80.16	78.16	82.84	83.24	0.204,258
175.....	0.99	1.01	1.10	1.12	1.19	49	66.76	70.00	72.44	77.52	81.12	0.208,300
Mean.....	0.97	0.91	0.95	1.00	1.04	51	67.04	75.29	77.43	85.64	87.38	0.246,405

Abbreviations as follows:—

La, Lb, etc..... Length of bundle in centimetres at A, at B, etc.
Fa, Fb, etc..... Fibre diameter in microns at A, at B, etc.

TABLE G.

Details of muscle bundles and muscle fibres.

Serial No. of Rabbit.	M. PSOAS MAJOR.							Area of Bundles in Cross-section (Sq. mm.).
	L.	No. of Fibres in Bundle.	Fa.	Fb.	Fc.	Fd.	Fe.	
Birth Group.								
93.....	2.1	88	9.22	9.51	9.48	9.45	9.99	0.006,277,1
94.....	2.4	83	9.26	8.34	8.48	8.35	8.81	0.004,877,5
95.....	2.6	98	8.84	8.20	8.19	9.62	9.24	0.005,987,6
96.....	2.6	85	7.45	7.43	7.59	7.01	7.70	0.003,695,4
97.....	2.5	103	8.30	7.80	8.23	7.57	7.75	0.005,087,2
98.....	2.9	92	9.28	8.86	8.92	8.05	8.21	0.005,418,9
100.....	2.6	87	8.42	9.06	7.98	8.71	9.85	0.005,291,5
101.....	2.8	98	8.77	8.11	8.09	8.05	8.24	0.005,238,7
102.....	2.4	86	8.73	7.61	8.02	7.90	7.97	0.004,377,1
103.....	2.4	95	9.10	8.99	8.79	8.52	8.96	0.005,870,3
Mean.....	2.53	92	8.74	8.39	8.38	8.32	8.67	0.005,212,1

Serial No. of Rabbit.	L.	Serial No. of Rabbit.	L.	Serial No. of Rabbit.	L.
100 Gm. Group.		150 Gm. Group.		220 Gm. Group.	
178.....	3.30	181.....	4.10	186.....	4.63
179.....	3.24	187.....	4.01	188.....	4.66
180.....	3.11	190.....	3.95	191.....	4.47
182.....	3.48	195.....	3.86	194.....	4.67
185.....	3.10	204.....	4.10	197.....	5.46
Mean.....	3.25	Mean.....	4.00	Mean.....	4.78

Serial No. of Rabbit.	L.	Serial No. of Rabbit.	L.
320 Gm. Group.		480 Gm. Group.	
183.....	5.94	192.....	6.63
193.....	6.30	198.....	7.30
196.....	6.03	200.....	7.72
199.....	6.18	202.....	7.64
201.....	5.93	203.....	7.77
Mean.....	6.08	Mean.....	7.41

Abbreviations as follows :—

L..... Length of bundles in centimetres.
Fa, Fb, etc..... Fibre diameter in microns at A, at B, etc.

TABLE G. (continued).

M. PSOAS MAJOR.

Serial No. of Rabbit.	L.	No. of Fibres in Bundle.	Fa.	Fb.	Fc.	Fd.	Fe.	Area of Bundles in Cross-section (Sq. m.m.).
600 Gm. Group.								
74.....	7.4	88	19.68	20.06	20.18	19.46	19.92	0.027,260
76.....	8.3	85	19.38	17.74	18.28	19.08	17.72	0.022,700
77.....	8.5	85	17.58	16.86	17.22	16.80	19.74	0.020,773
78.....	7.9	78	20.40	19.06	19.20	18.26	19.30	0.022,678
79.....	8.1	90	20.64	19.38	18.38	19.78	18.10	0.026,221
80.....	8.2	101	18.46	18.36	17.48	18.42	18.08	0.026,160
81.....	7.8	99	19.70	20.24	21.40	20.62	19.84	0.032,232
82.....	8.1	85	23.10	20.66	20.48	19.62	20.04	0.028,827
83.....	8.4	88	22.52	20.78	20.16	19.38	20.52	0.029,415
85.....	8.5	110	21.38	21.08	20.60	20.18	20.40	0.037,126
Mean....	8.1	91	20.28	19.40	19.34	19.16	19.37	0.027,339
1,200 Gm. Group.								
62.....	7.3	79	33.32	30.62	29.32	30.48	28.52	0.057,530
63.....	8.1	92	32.70	29.14	28.02	28.12	32.14	0.065,814
64.....	10.4	105	29.62	27.72	28.02	25.78	26.80	0.062,775
65.....	9.3	95	34.80	30.72	31.16	27.60	28.10	0.069,318
68.....	10.1	81	31.12	28.50	27.84	27.58	28.36	0.052,328
69.....	10.3	87	30.32	28.18	27.00	26.62	27.92	0.053,600
87.....	11.0	80	36.38	30.80	31.02	29.84	32.84	0.065,066
88.....	11.4	96	29.70	26.54	24.96	25.24	27.14	0.053,831
89.....	7.3	84	26.94	24.46	27.00	26.78	27.90	0.046,751
90.....	9.9	72	29.32	30.00	28.66	28.82	29.74	0.048,580
Mean....	9.5	87	31.42	28.67	28.30	27.69	29.03	0.057,560
1,800 Gm. Group.								
43.....	11.7	75	34.50	34.94	32.22	33.24	32.72	0.066,185
44.....	12.4	93	37.86	40.32	34.78	33.06	35.10	0.095,823
48.....	11.3	76	38.08	37.92	34.96	38.50	38.96	0.084,747
50.....	11.0	98	36.94	38.78	38.90	38.68	40.50	0.115,634
51.....	11.9	110	41.22	40.04	40.08	36.78	41.90	0.138,230
52.....	9.2	105	39.38	36.96	36.48	36.24	37.72	0.115,105
53.....	11.4	94	45.74	43.64	44.58	41.86	41.72	0.139,765
54.....	10.9	87	41.00	36.02	35.62	37.90	42.60	0.101,967
55.....	9.8	116	38.92	38.24	35.86	36.44	47.48	0.127,368
58.....	11.7	93	41.38	35.06	33.50	37.72	36.82	0.099,455
Mean....	11.1	95	39.50	38.19	36.70	37.04	38.55	0.108,428

Abbreviations as follows:—

L..... Length of bundle in centimetres.

Fa, Fb, etc..... Fibre diameter in microns at A, at B, etc.

TABLE G. (*continued*).

Serial No. of Rabbit.	M. Psoas Major.							
	L.	No. of Fibres in Bundle.	Fa.	Fb.	Fc.	Fd.	Fe.	Area of Bundle in Cross-section (Sq. mm.).
2,400 Gm. Group.								
38.....	12.5	103	46.38	43.54	42.50	38.72	44.46	0.150,413
40.....	13.0	92	43.58	41.06	40.28	39.64	39.70	0.120,577
41.....	13.9	88	44.36	41.40	37.60	37.18	43.36	0.114,939
42.....	11.1	91	48.90	42.82	42.70	46.02	48.24	0.149,529
47.....	10.4	80	45.40	39.56	42.00	41.44	40.46	0.109,625
49.....	12.4	86	42.32	45.22	39.30	41.12	43.38	0.120,685
60.....	12.7	100	47.60	41.88	43.42	43.90	46.80	0.157,071
61.....	13.8	84	44.44	41.06	39.78	41.40	45.30	0.118,605
75.....	14.5	92	42.22	43.42	42.20	43.60	44.36	0.134,599
86.....	13.2	91	44.74	44.52	45.92	49.52	49.16	0.156,339
Mean.....	12.8	91	44.99	42.45	41.57	42.25	44.52	0.133,238
3,000 Gm. Group.								
39.....	13.5	87	54.62	46.56	45.94	42.36	50.14	0.156,908
59.....	14.1	93	50.58	45.78	46.40	46.26	49.28	0.166,914
66.....	14.4	109	48.54	46.20	44.12	48.54	47.36	0.188,707
109.....	13.6	87	41.90	37.34	39.28	41.46	41.78	0.111,249
112.....	13.7	109	51.36	43.62	40.56	44.18	47.80	0.177,231
150.....	14.6	90	43.58	41.50	42.54	43.98	49.54	0.138,288
157.....	12.6	94	44.26	40.04	44.60	45.38	47.64	0.145,410
170.....	14.2	84	43.98	45.86	46.66	46.08	48.52	0.140,939
172.....	13.1	103	47.04	49.16	44.26	46.74	48.80	0.180,224
176.....	14.5	86	43.14	39.76	40.90	42.66	44.76	0.120,514
Mean.....	13.8	94	46.90	43.58	43.53	44.76	47.56	0.152,538
Mature Group.								
108.....	13.2	89	49.76	45.88	46.66	43.62	54.74	0.161,925
110.....	15.3	86	51.04	47.44	46.98	51.60	52.86	0.168,726
111.....	13.0	98	45.68	43.40	41.38	45.72	46.70	0.152,967
158.....	13.6	82	54.52	46.52	44.70	48.20	47.86	0.150,618
159.....	13.8	79	51.20	50.54	48.28	48.38	49.94	0.153,076
168.....	13.4	84	44.02	45.92	45.92	43.50	48.60	0.137,123
171.....	13.7	80	51.34	46.96	50.44	49.06	56.12	0.162,019
173.....	13.7	88	45.94	44.46	42.66	42.20	44.38	0.133,625
174.....	13.7	100	46.68	46.02	48.12	45.98	52.82	0.180,354
175.....	15.1	88	46.62	44.98	44.30	42.94	45.46	0.139,089
Mean.....	13.9	87	48.68	46.21	45.96	46.12	49.95	0.153,952

Abbreviations as follows:—

L..... Length of bundle in centimetres.

Fa, Fb, etc..... Fibre diameter in microns at A, at B, etc.

CHAPTER VIII.—RELATIVE GROWTH.

In an attempt to derive a bio-mathematical concept of the growth phenomena, Mr. van der Reyden, Section of Statistics, has made a considerable study of the wealth of data accumulated during the experiment. His findings will be presented at some future date, but many interesting points have already been brought out by his researches.

As it would be undesirable not to mention this aspect, Mr. van der Reyden has kindly consented to summarise certain findings for inclusion in this work. For convenience, his discussion is treated in a separate section. This addition enhances the value of the study and may be of service to other workers in this field.

RELATIVE GROWTH.

By D. VAN DER REYDEN, Section of Statistics,
Onderstepoort.

In this chapter the quantitative methods used in evaluating the observed data are discussed shortly. The scope and significance of the law of simple allometry will be emphasised. Statements about the inter-relation of weight and form will lead to the question of what constitutes appropriate measurements in muscle growth.

PART I.—The Law of simple Allometry.

(a) Mathematical formulation.

Proposed by Julian S. Huxley in 1924, the law of simple allometry, in the sense of heterauxesis as well as allomorphosis (Huxley, Needham, and Lerner, 1941), has been extensively used to relate the measurement of the part to the measurement of the whole or standard. It is written

$$y = bx^a \dots\dots\dots (1)$$

where—

- y = measurement of part,
- x = measurement of whole,
- b = growth index, the value of y when $x = 1$,
- a = equilibrium constant.

The important parameter is a , which can be written

$$a = \frac{\text{relative rate of increase of } y}{\text{relative rate of increase of } x} \dots\dots\dots (2)$$

which, on integration, yields expression (1). Differentiating the expression we find the instantaneous rate of change to be

$$\frac{dy}{dx} = \frac{ay}{x} \dots\dots\dots (3)$$

Expressing the allometric equation in logarithms the linear form is obtained,

$$\log. y = \log b + a \log. x \dots\dots\dots (4)$$

(b) *Statistical formulation.*

(1) *The estimation problem.*—It is the task of statistics to investigate the possibility of bridging the gap between the mathematical formulation and the observed data. Most data to which the allometric equation is fitted can be expressed symbolically in either of the two forms presented below.

TABLE 35.
Observational data.

TIME CLASSIFICATION.			CLASSIFICATION IN TERMS OF WHOLE.	
Time Point.	Measurement of Part.	Measurement of Whole.	Measurement of Whole.	Measurement of Part.
1	$\begin{matrix} Y(1, 1) \\ Y(1, 2) \\ \vdots \\ Y(1, k) \end{matrix}$	$\begin{matrix} X(1, 1) \\ X(1, 2) \\ \vdots \\ X(1, k) \end{matrix}$	X_1	$\begin{matrix} Y(1, 1) \\ Y(1, 2) \\ \vdots \\ Y(1, k) \end{matrix}$
2	$\begin{matrix} Y(2, 1) \\ Y(2, 2) \\ \vdots \\ Y(2, k) \end{matrix}$	$\begin{matrix} X(2, 1) \\ X(2, 2) \\ \vdots \\ X(2, k) \end{matrix}$	X_2	$\begin{matrix} Y(2, 1) \\ Y(2, 2) \\ \vdots \\ Y(2, k) \end{matrix}$
n	$\begin{matrix} Y(n, 1) \\ Y(n, 2) \\ \vdots \\ Y(n, k) \end{matrix}$	$\begin{matrix} X(n, 1) \\ X(n, 2) \\ \vdots \\ X(n, k) \end{matrix}$	X_n	$\begin{matrix} Y(n, 1) \\ Y(n, 2) \\ \vdots \\ Y(n, k) \end{matrix}$

The first symbol in the bracket refers to the classification point, while the second refers to the animal in each subgroup of k animals. Excluding the case in which the same individual can be measured consecutively, arithmetical averages of each subgroup are taken, thus:—

TABLE 36.
Average Figures.

Whole.	Part.	Estimated Part.
x_1	y_1	\hat{y}_1
x_2	y_2	\hat{y}_2
\vdots	\vdots	\vdots
x_n	y_n	\hat{y}_n

It is now assumed that the allometric formulation is applicable and the statistical theory of estimation [Fisher, 1938; 1941] brought into play to obtain estimates of the parameters b and a . Attention must also be drawn to a publication by Kavanagh and Richards (1942) in which various methods of curve fitting are discussed. Utilising the obtained estimates of the parameters the third column in Table 36 can be calculated.

(2) *Confidence intervals and applicability of allometric law*.—Very closely allied with the process of estimation is the question of goodness of fit of the equation. Statistical literature abounds with different methods, ranging from the χ^2 -test first proposed in 1900 to the Theory of Runs (Mood, 1940). Most of these have as starting point the assumption that the differences between the observed and estimated values of y are due to chance. While not stating that the reasoning involved in any one of these methods is of a *petitio principii* nature, the need for a method independent from the estimated values is evident. This requirement is partly met by the theory of *confidence intervals* (Neyman, 1941, where an extensive bibliography is given) or, for that matter, by Fisher's *fiducial theory* (1936, 1942).

As applied to the test for allometry, the reasoning is shortly as follows:—

From a statistical point of view the allometric equation, if appropriate, will hold between the "true" values of y and x . In how far can \hat{y} be regarded as a "true" value of y ? That is, how big a deviation can be allowed between y and \hat{y} ? As the y -values are averages, calculated on k replicates at each measuring point of the whole, it is possible to determine limits between which the unknown "true" value at each point almost certainly lies.

Consider the k replicates, Y_1, Y_2, \dots, Y_k , at any point as independent variables, varying normally about η , the "true" value, with an unknown standard error σ . Then confidence interval for η is given by

$$y - s_y t_{\beta}(k) \leq \eta \leq y + s_y t_{\beta}(k) \dots \dots \dots (5)$$

where $y = \frac{1}{k} \sum Y$, $s_y^2 = \frac{1}{k(k-1)} \sum (Y - y)^2$, and $t_{\beta}(k)$ is Fisher's t corresponding to the number of degrees of freedom $(k - 1)$ and to chosen confidence coefficient β .

In other words, if the true value lies within the confidence interval determined by the data and the significance test (5), that value is not contradicted by the data at the level of significance chosen. By calculating at each point a confidence interval for the arithmetic mean of the part, and by connecting the end-points of consecutive intervals a *confidence region* for the experiment is obtained. If now the allometric formulation is *not* applicable, its curve will intersect the boundary lines of the confidence region at one or more points. It must be emphasised that if the allometric curve lies within the confidence region, it does not necessarily follow that it is "true". In conformity with modern scientific methodology, statistics cannot prove a statement correct; it can only reject an incorrect statement.

It is not intended to assess the various biological interpretations existing especially about the exponent a in the allometric formulation. Emphasis is rather laid upon the methodological aspect. Two basic requirements must first be satisfied before it is attempted to assign a biological meaning to the so-called equilibrium constant. The first is that the measurement performed

on the part or whole must *in itself* be capable of biological explanation. Secondly a valid criterion must be available to control the acceptance, on the basis of the observed data, of the allometric formulation. These two requirements have not yet received the attention they merit.

PART 2.—Body Weight as Standard.

Let y be the measurement of the part and x that of the whole, in this experiment the weight of the rabbit. For computational purposes expression (1) was formulated as

$$\hat{Y} = B + AX \dots \dots \dots (6)$$

where—

\hat{Y} = best estimate of $Y = \log. y$,

X = $\log. x$,

B = $\log. b$,

A = best estimate of a .

As graphical analysis showed that some of the measurements did not obey (6), it was decided as a preliminary step to fit to all measurements a logarithmic parabola as first empirical extension of the allometric formula, thus

$$\hat{Y} = B + AX + CX^2 \dots \dots \dots (7)$$

as equivalent of

$$\hat{y} = bx^a 10^{c \log. x} \dots \dots \dots (8)$$

$$\text{or } \hat{y} = bx^a + c \log. x \dots \dots \dots (9)$$

Curve fitting was done by the method of least squares. As the same set of body weights was used throughout the experiment, Fisher's technique (1941) was used to avoid the simultaneous equations arising on each occasion. Further simplification became possible by introducing Waugh's method of solving the equations (1935). After obtaining the numerical values of the constants B , A and C , last-mentioned was tested for significance. If insignificant, the last term in (7) was omitted and the necessary corrections applied to A and B . For instance, the weight of *M. Psoas* before correction was expressed as

$$\hat{Y} = -3.715 + 1.535X - 0.0361X^2.$$

After correction it became

$$\hat{Y} = -3.459 + 1.338X.$$

Finally the confidence regions were calculated. Control was established at the 1 per cent. level of significance.

Instantaneous rates of increase relative to body weight were obtained by mathematical differentiation of the allometric formula or expression (7). The formulae became respectively

$$\frac{dy}{dx} = A \frac{\hat{y}}{x} \dots \dots \dots (10)$$

and

$$\frac{dy}{dx} = (A + 2CX) \frac{\hat{y}}{x} \dots \dots \dots (11)$$

TABLE 37.
Estimates of relative growth parameters.

Characteristic.	M. GASTROCNEMIUS.					M. PSOLAS.				
	B.	b.	A = a.	C.	I.*	B.	b.	A = a.	C.	I.*
Weight.....	-5.5016	0.000,003	2.71	-0.289	D	-3.4592	0.003,474	1.34	—	O.
Length.....	-1.7474	0.017,890	2.16	-0.9366	Q	-0.9366	0.115,720	0.91	-0.086	O.
Width.....	-1.5141	0.030,612	0.48	—	D	-1.4647	0.034,301	05.0	—	O.
A.....	-1.3879	0.040,935	0.50	—	D	-1.3804	0.043,611	0.49	—	O.
B.....	-1.3443	0.045,259	0.49	—	D	-1.3262	0.047,185	0.49	—	O.
C.....	-1.4483	0.035,621	0.51	—	D	-1.3183	0.048,051	0.50	—	D.
D.....	-1.6047	0.024,848	0.51	—	D	-1.2928	0.050,956	0.48	—	O.
E.....										
Depth.....	-1.8322	0.014,716	0.44	—	D	-2.7363	0.001,835	0.60	—	O.
A.....	-2.8903	0.001,317	1.38	-0.189	O	-2.4071	0.003,917	0.54	—	O.
B.....	-2.4877	0.003,407	1.08	-0.133	O	-2.2343	0.005,830	0.52	—	O.
C.....	-1.7184	0.019,125	0.41	—	D	-2.1237	0.007,521	0.50	—	O.
D.....	-1.9866	0.010,314	0.43	—	D	-2.0208	0.009,532	0.49	—	O.
E.....										
Bundle length.....	—	—	—	—	—	-1.0908	0.081,133	1.01	-0.108	O.
Bundle size.....	-4.2112	0.000,061	1.04	—	O	-4.0359	0.000,092	0.92	—	O.
Fibre Diameter.....	0.2710	1.8664	0.44	—	O	0.0666	1.1657	0.46	—	O.
A.....	0.1417	1.3858	0.50	—	O	0.0571	1.1405	0.46	—	O.
B.....	0.0913	1.2340	0.52	—	O	0.0632	1.1666	0.46	—	O.
C.....	0.0879	1.1426	0.54	—	O	0.0433	1.1049	0.46	—	O.
D.....	0.0754	1.1896	0.53	—	O	0.0404	1.0975	0.47	—	O.
E.....										

* Abbreviation :-I = Intersecta.

Results:—

In Table 37 the results of the curve fittings are shown. Under column headings A and C only those values which showed significance by the t-test are shown. To test agreement between the ordinary significance tests and the confidence region, it was arbitrarily decided to employ two or more intersections of the fitted curve with the boundaries of the confidence region as a criterion of "badness" of fit. This is symbolically represented by "D" in the table, while "O" stands for no or one slight intersection.

A study of the results reveals:—

(1) With the exception of muscle and bundle length *M. Psoas* grows heterauxetically in all its measurements; *M. Gastrocnemius* does so only in the measurements of its lesser constituents, size of bundle and fibre diameter.

(2) This fact makes an inter-muscle comparison of the equilibrium constants very difficult.

(3) On the basis of the available measurements no evidence can be presented for the existence of growth centres and gradients within the muscle, excepting fibre diameter of *M. Gastrocnemius* and muscle thickness (depth) of *M. Psoas*.

(4) Intra-muscularly it seems that the equilibrium constants of muscle width and fibre diameter are related.

Figures 24-30.

These figures are presented as a matter of interest. They illustrate that a linear relationship in logs can be established for many measurements by the method of least squares, control being established at the 5 per cent. level of significance. However, when the confidence regions are calculated, intersection of the fitted line with the boundaries of the confidence region indicates a "badness" of fit for many of these measurements (two examples demonstrated in Figures 32 and 33).

The rapidly growing literature contains numerous similar examples of curve-fitting. Either the least squares method is employed to establish a linear relationship in logs, or the data may even be fitted by eye from a scatter diagram. By rejecting certain proposed curves the confidence region criterion assists in testing agreement with the ordinary significance tests, as well as eliminating a possible degree of wishful thinking.

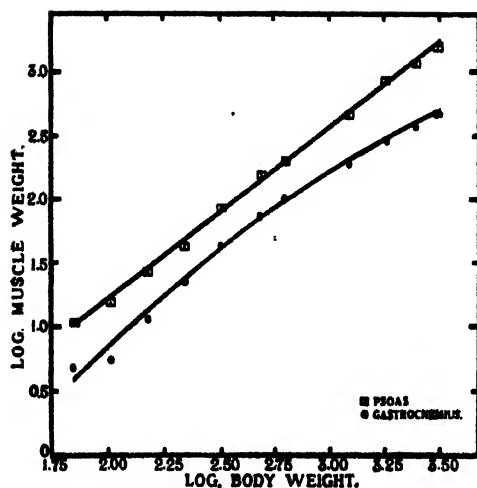


Fig. 24.—Weight of Muscle.

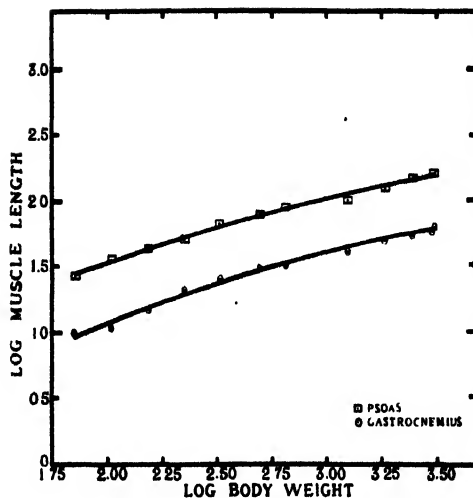


Fig. 25.—Length of Muscle.

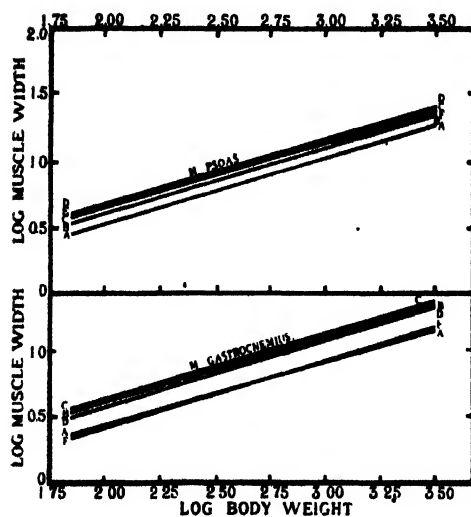


Fig. 26.—Width of Muscle.

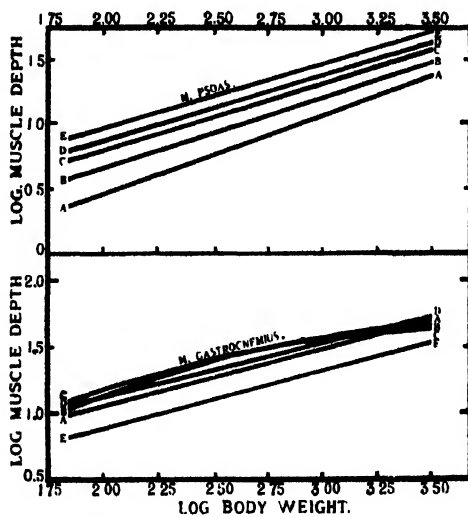


Fig. 27.—Depth of Muscle.

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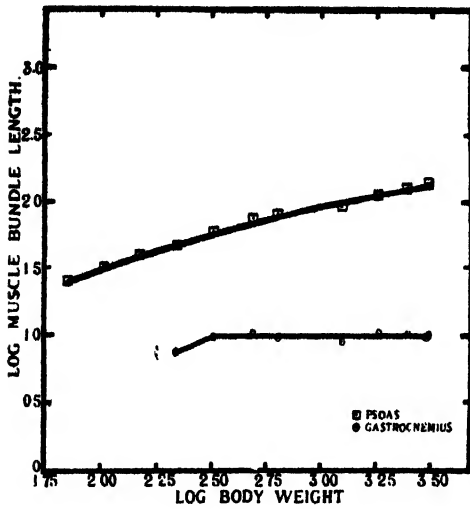


Fig. 28.—Length of Muscle Bundle.

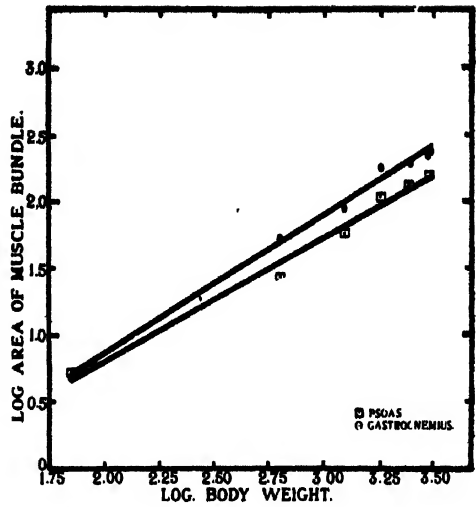


Fig. 29.—Thickness of Muscle Bundle.

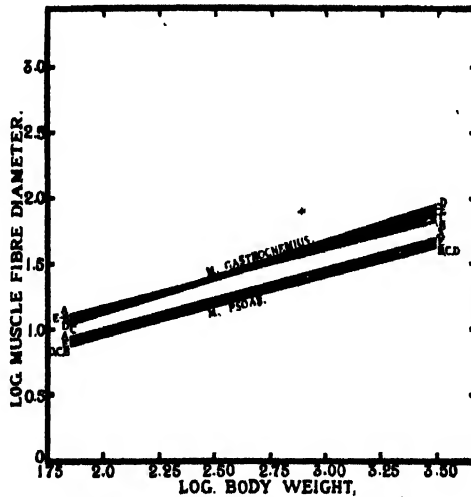


Fig. 30.—Diameter of Muscle Fibre.

Figures 31-33.

In Figure 31 the confidence region criterion supports the straight-line relationship in logs., established by the least squares method. In Figures 32 and 33 the fitted line intersects the boundary of the confidence region, indicating the "badness" of fit for width and depth of the Gastrocnemius muscle at site A.

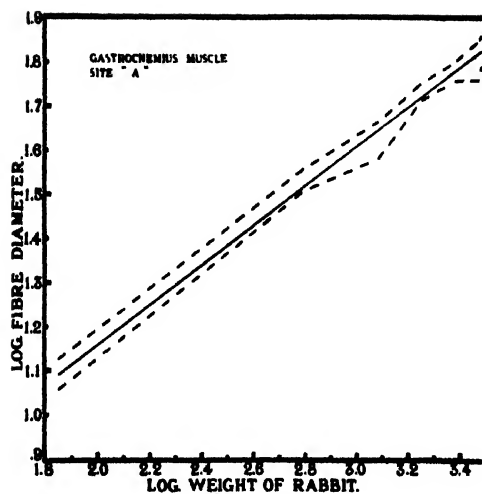


Fig. 31.—Fibre Diameter—M. Gastrocnemius, Site A.

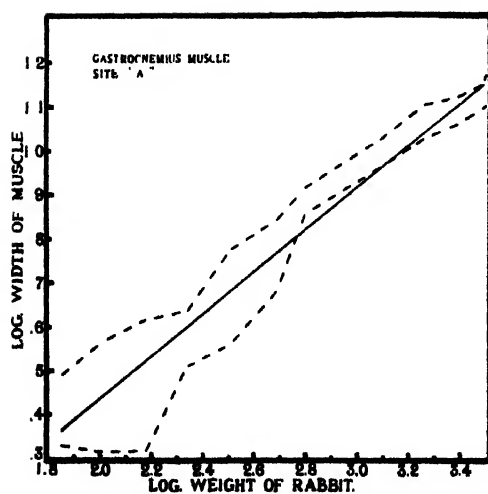


Fig. 32.—Width—M. Gastrocnemius, Site A.

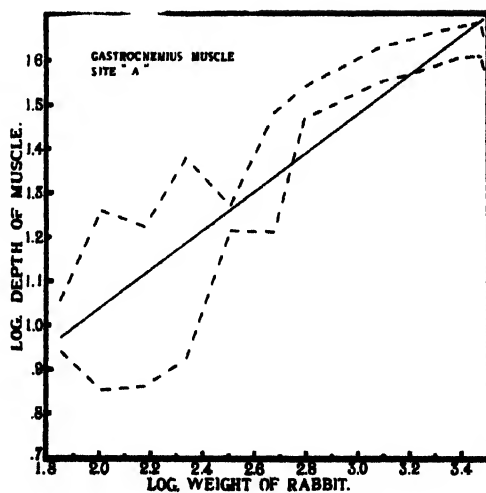


Fig. 33.—Depth—M. Gastrocnemius, Site A.

PART 3.—Muscle Weight in Terms of Linear Measurement.

In how far, if at all, is it possible to express weight growth in terms of increase of linear measurements, length, width, and depth? Before attempting to answer this question, it must first be investigated whether the weight at any development-point can be approximated by some combination of length, width and depth.

Assuming that for our purposes weight is equivalent to mass, a relationship between weight and volume can be written

$$(\text{volume}) = (\text{mass}) \times (\text{density})^{-1} \dots \dots \dots (12)$$

The next step would be to express volume in terms of the linear measurements. As length was taken from origin to insertion, *extra animalis*, and the widths and depths perpendicular to this line, a cuboid form suggests itself as a first approximation. In fact, enforces itself due to the method of measurement.

As practical density determinations were only made on five weight groups, it was deemed advisable to restrict all attempts at "muscle-building" to these. In Table 38 is shown the actual density figures as well as the hypothetical ones, calculated on a cuboid assumption.

TABLE 38.

Density Calculations.

Weight Group.	GASTROCNEMIUS.		PSOAS.	
	Actual.	Hypothetical.	Actual.	Hypothetical.
Gm.				
100.....	1.05	1.31	1.06	1.69
150.....	1.06	1.46	1.05	1.71
220.....	1.06	1.41	1.06	1.74
320.....	1.06	1.39	1.06	1.53
480.....	1.06	1.21	1.06	1.46

As the hypothetical density figures are much too large, it means that the calculated volumes are too small. This in turn can only signify that the shape of the muscle cannot be represented as a cuboid. Various other formulations were tried: addition of smaller cuboids, solids of revolution, etc., but all these methods yielded values still worse.

It can only be concluded that the volume or shape of the muscle cannot be approximated by an arbitrary system of linear measurements perpendicular to one another. Only when a fixed reference system can be found, inside or outside the muscle, and measurements, linear or non-linear, appropriate to the shape of the muscle performed, will this morpho-metrical problem be capable of solution.

It must be pointed out that it is possible interpolationarily to express muscle weight in terms of any of the linear measurements. By utilising the results from Table 37 we have symbolically

$$\left. \begin{aligned} W &= f_1(x) \\ L &= f_2(x) \\ B &= f_3(x) \\ D &= f_4(x) \end{aligned} \right\} \dots\dots\dots (13)$$

where—

x = body weight,
 W = muscle weight,
 L = length,
 B = width,
 D = depth.

By mathematically eliminating body weight between any two of the above equation the following permutations could easily be obtained.

$$\left. \begin{aligned} W &= F_1(L) \\ W &= F_2(B) \\ W &= F_3(D) \end{aligned} \right\} \dots\dots\dots (14)$$

It might be thought that by differentiating W with respect to L , then to B and finally to D , and comparing the three sets of relative increases an answer might be given to the question whether increase in muscle weight is mainly "caused" by increase of any one of the three linear measurements. This supposition is quite wrong for the question itself has no meaning. Growth of muscle is a three-dimensional affair, and the increase in weight is due to the *simultaneous* increase in three dimensions, as has been clearly shown in preceding chapters. A practical illustration might be of interest, however. It is possible to write the instantaneous rates of increase of weight of *M. Psoas*, say, relative to the linear measurement as

$$\left. \begin{aligned} \frac{dW}{dL} &= \frac{dW/dx}{dx/dL} = \frac{A_w}{A_1 + 2c \log x} \cdot \frac{W}{L} \\ \frac{dW}{dB} &= \frac{dW/dx}{dx/dB} = \frac{A_w}{A_b} \cdot \frac{W}{B} \\ \frac{dW}{dD} &= \frac{dW/dx}{dx/dD} = \frac{A_w}{A_d} \cdot \frac{W}{D} \end{aligned} \right\} \dots\dots\dots (15)$$

where A is the practical equilibrium constant.

It has already been stressed that the system of linear measurement utilised in this experiment only allows a cuboid reconstruction of the muscle. That is, we are entitled to write

$$W = \rho LBD \dots\dots\dots (16)$$

or some such expression.

If this is substituted for W in the above expressions, it can be seen at once that the rate of increase of W with respect to either L or B or D depends on the magnitudes of *both* the remaining two at the *same* time.

It might be useful to point out that if $L > B > D$ then $\frac{dW}{dL} < \frac{dW}{dB} < \frac{dW}{dD}$.

Summing up, it can be stated however useful length, width, or depth might be considered *separately*, they are of little or no use in evaluating the weight of the muscle. Taking a mechanical analogy, one might investigate the utilitarian value of the length, width, and depth of an electrical dynamo relative to its efficiency.

Conclusion.

In the introduction to Chapter 1 it was stated "Definition of the quantitative character of muscle, in terms of measurable biological entities such as muscle bundle and muscle fibre, constitutes a primary requisite for such investigation."

Translated into statistical language it may be stated that the measurements performed for the purpose of throwing light upon muscle growth must be of such a nature as to satisfy the following requirement:

Measurement of individual muscle = measurement of lesser muscle unit
X number of lesser units.

As this statement opens up vast fields of investigation, details will be omitted. Suffice it to state that powerful statistical procedures are available for simplification, so as to allow an unambiguous interpretation of data based on biological fact.

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Meat Studies No. 2.—Toughness of Meat.

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SECTION 1.—INTRODUCTION.

QUALITIES such as flavour, texture, juiciness, colour, and aroma contribute to the appreciation of meat. However, most people agree that tender meat is good meat. Toughness makes for unpalatability, or even inedibility for a section of the population.

Agreement has not been reached regarding the nature of toughness, although the three constituents of meat—muscle fibres, connective tissue, and fat—must each play a rôle. Evidence is still inconclusive as to the exact relationship of the various factors contributing to toughness. These factors are as follows (Sweetman, 1937):—

1. *The quality of muscle fibres.*

Toughness of the muscle fibre depends upon the development and density of the fibre, possibly due to changes occurring with age, activity, etc.. In the past, attention has been directed mainly to the connective tissue content of meat, and quality of fibre has received scant attention. While the quality of any foodstuff is difficult to define, the work of Bate-Smith (1934, 1935), determining the proteins of muscle is a big step towards a precise definition of quality in the case of meat. Unfortunately this lead has not yet been followed up.

2. *The kind and amount of connective tissue.*

Toughness due to connective tissue depends upon the proportion of collagen and elastin present in the meat. In general, elastin constitutes only an insignificant fraction of the connective tissue. As heat has little action on elastin it is unchanged during cooking. On the other hand, heat changes collagen to gelatin which is water-soluble and tender. Furthermore, collagen softens and swells under the action of very dilute acid, thereby facilitating the process of heat change. As the lactic acid normally produced in flesh reaches a concentration of 0.9 per cent. two to three days after slaughter, this is an important consideration.

3. *The amount and distribution of the fat.*

Toughness tends to be diminished by fat deposition within a muscle. Presumably the infiltration of fat between the collagen fibres separates these fibres, which can then be broken down more easily by the preparation of the meat and by chewing.

4. *The texture or "grain" of the meat (size of muscle bundle).*

A coarse texture is usually associated with poor eating qualities (stringy or tough meat). Hammond (1940) is of opinion that the size of the muscle bundles is the main factor affecting tenderness in meat.

5. *The degree of ripeness of the meat.*

During rigor mortis the previously soft and flabby muscle fibres become firm and taut. Meat eaten in this condition is rubbery, but after the resolution of rigor the muscles are soft and more tender. A further noticeable improvement is brought about by an additional period of ripening. Almost certainly the increasing tenderness during ripening must be partly due to a decrease in collagen, but an investigation of the physical consistence of the proteins in the muscle fibre during these changes is also highly desirable.

SECTION 2.—MEASUREMENT OF TOUGHNESS.

A tasting panel may be formed to determine the final quality of the meat. Here selected persons taste a number of meat samples, and grade each sample on a scale of points for each of its properties (tenderness, texture, flavour, etc.). According to the score averaged for specific properties the samples can be rated, in order from best to poorest.

In addition, various mechanical penetrating or cutting devices may be used to grade meat. Owing to the difficulty of establishing an efficient tasting panel these objective methods of measuring tenderness have many advantages. On the other hand, it is almost impossible to imitate the complex cutting and grinding action performed by the teeth, so that these instruments are not entirely reliable in interpreting human taste preferences.

Among the instruments devised for testing meat toughness are the following:—

1. *Tressler and Murray's penetrometer.*

By subjecting the meat to the action of a penetrometer a measurement is obtained of the pressure necessary to force the point of the instrument into the meat. A high reading indicates that great force is required, i.e., toughness.

2. *Warner-Bratzler shear.*

A shearing device has been developed by the United States Bureau of Animal Industry for measuring tenderness. The instrument measures in pounds the shearing resistance of a one-inch sample core, cut with the grain of the meat by means of a sharpened steel cylinder similar to a cork-borer.

3. *Volodkevich's chewing apparatus.*

This apparatus, mainly in use in Germany, records the force required to crush meat between two wedges. The assumption is that the value obtained reproduces the chewing resistance of the meat, and serves as a measure of its tenderness.

4. *Winkler's apparatus.*

Winkler (1939) constructed an apparatus somewhat similar to that of Volodkevich, of simple design, with the advantage of a recording device.

Chemical methods have also been used for comparing meat quality. Unfortunately attention has largely been focussed on connective tissue to the exclusion of muscle proteins. By the chemical determination of the collagen and elastin content it becomes possible to compare different types of muscle, as well as different classes of animals. Lately, workers have determined the amount of collagen changed to gelatin during cooking, to indicate the probable tenderizing effect of different types of cooking. With this method of approach only one phase of the toughness problem is examined, that of connective tissue. The other equally important phase, that of physical consistence of muscle protein, is not considered.

SECTION 3.—FACTORS INFLUENCING TOUGHNESS OF MEAT.

1. *Muscle Fibres.*

Meat in rigor mortis is generally believed to be less tender than meat taken from the carcass immediately after slaughter and cooked at once. (Brewster, 1944. pages 140-141, 143, 144.) This is due to a hardening and stiffening of muscles during rigor, as a result of the post-mortal coagulation of muscle proteins. The magnitude of this toughening effect can probably be deduced from measurements of the increased hardness of muscle in rigor. Thus, Mangold, cited by Paul *et al* (1944), observed a relative increase in stiffness of muscle during rigor of approximately 67 per cent., as compared

with muscle immediately after death. Bate-Smith (1939) showed that "in the Psoas of the rabbit, the onset of rigor is marked by a change in the modulus of elasticity from a value between 700 and 3,000 to one in the neighbourhood of 10,000".

After the resolution of rigor, when the muscles again become flabby, the meat is more tender. For instance, chickens killed and stored overnight are more tender than chickens cooked and eaten on the day of killing (Lowe, 1937). Hanson *et al* (1942) too, find that meat from chickens cooked shortly after killing is rubbery and difficult to chew. Paul *et al* (1944) state that beef roasts were "extremely difficult to cut when rigor was at its peak, as they were hard and rigid and the procedure was similar to cutting a rubber cork".

Toughness of the muscle fibre is regarded as being dependent upon the development and density of the fibre, due to activity and to changes occurring with age. Inherent differences are known to exist in the density of the muscle fibres in different types of muscle and animal. Thus Beard's (1924) histological study showed that, in the toughest muscles the fibres contain the densest sarcoplasm, whereas in the tenderest muscles the fibres contain the lightest sarcoplasm.

Volodkevich (1938) established age differences in the density of muscle fibres. After a force of eleven kilograms had been attained in his apparatus, the resistance across the grain of beef decreased slightly, subsequently increasing markedly when the wedges were close together. With veal this second increase in resistance was absent. His explanation is that the more tender fibres are torn early in the squeezing process, while the stronger, tougher fibres which remain resist this force towards the end of the process.

When meat is subjected to mechanical tests for toughness the direction of the grain of the sample is of importance. If the squeezing or cutting force is applied transverse to the line of the fibres, resistance is offered mainly by the fibres. However, when this action is parallel to the fibres the resistance is derived mainly from connective tissue. Steiner (1939) showed that this latter force is not reduced by an increased period of holding the meat nor by an increased temperature, whereas a marked reduction occurs in the force required to cut through the muscle fibres. He holds that connective tissue plays a negligible rôle in the great reduction of toughness brought about in this way. Rather, there is a change in the muscle fibres which become less dense and are softened.

In addition to the naturally occurring changes, the effect of cooking must also be considered. Moran and Smith (1929), Steiner (1939), Ramsbottom *et al* (1945) hold that raw meat is more tender than cooked meat. A contrary finding is presented by Black *et al* (1931), who obtained results showing that the shearing strength of cooked meat is less than of uncooked meat. Presumably the initial effect of heating is to increase toughness by coagulating the muscle protein, but Steiner observes that the longer cooking is continued the more tender is the final product.

Gottschall and Kies (1942) consider that in the absence of adequate criteria of what constitutes tenderness it is difficult to determine quantitative tenderization of meat. Proteolytic enzymes induce tenderness by virtue of their action in breaking down protein in ripening meat, but Hoagland *et al* (1917) found that the changes in the protein constituents are almost negligible in the case of meat stored for three weeks.

2. The Connective Tissue Content.

Differences in connective tissue content have been shown for different species of animals. Pork, for instance, contains little connective tissue. For the different cuts of pork, the percentage of connective tissue is moreover nearly similar, as compared with a wide variation in the different cuts of beef (Mitchell *et al.*, 1927). In beef, Mitchell *et al.* (1928) found that the less tender cuts (shoulder) contain more collagen than the more tender ones (rib, tenderloin).

This observation is confirmed by Ramsbottom *et al.* (1945), who show that muscles containing little connective tissue are more tender than muscles containing large amounts of connective tissue.

It is well known that coarse cuts with large amounts of connective tissue become relatively more tender after hanging than tender cuts containing less connective tissue (Moran and Smith, 1929; Steiner 1939). Observations by Hall and Mackintosh (1930-35) indicate that when beef cuts are ripened the collagen content may change very little when this is originally less than four per cent. When originally eight to ten per cent. of collagen is present this may be reduced one-half and correspondingly less between four and eight per cent.

Contradictory results are found regarding the amount of connective tissue present in animals of increasing age. Observations by Hammond (Moran and Smith, 1929, p. 42) show that the proportion of connective tissue to muscle substance is considerably higher in the tender meat of foetal lamb than in the tougher meat of adult sheep. This finding is corroborated for the rate by Hines and Knowlton (1939). These authors calculate that the connective tissue decreases from forty per cent. of the total muscle mass at fifteen days of age to fifteen per cent. at ninety days. On the other hand, Mackintosh *et al.* (1936) obtained a higher collagen value in mature steers than in yearling steers. This was associated also with an increase in shear value. Mitchell *et al.* (1932-33) too, report that the connective tissue content of lean meat from choice steer calves may be less than that from carcasses of choice yearling steers.

3. The Amount and Distribution of Fat.

Fattening beef animals brings about a relative increase in tenderness of the Longissimus dorsi muscle of about thirty per cent. (Helser *et al.*, 1930), so it is to be expected that tenderness increases with increase in the grade of carcass in most animals (Stanley and Cline, 1929). Mackintosh *et al.* (1931-35, 1936) show that changes in tenderness are associated with the degree of marbling of the meat, so that an increased finish tends to render meat more tender. In general, tenderness of rib roasts shows a tendency to increase with increasing finish (U.S. Conf. Co-op. Meat Investigations, 1937). Mutton chops from fattened ewes, old and young alike, graded higher in tenderness than cuts from old thin animals (Eckblad and Cline, 1936). More fat was present in the meat of cattle fed a supplement of corn in addition to Lespedeza hay, than in cattle receiving Lespedeza hay alone, and the shearing test showed an increased tenderness of more than thirty per cent. in the meat of the supplemented animals (Report B.A.I., 1941).

In the face of these studies there would seem to be little reason for doubting that fatness increases tenderness. Nevertheless, careful work by Cover *et al.* (1944) indicates that it is doubtful whether fatness influences

tenderness in lamb to any marked extent. Working with beef, Ramsbottom *et al* (1945) too, find no relationship between the amount of fat within a muscle and the toughness of that muscle.

4. The Texture or "Grain" (size of muscle bundles).

Texture and consistence of meat have long been used by the trade as indications of quality. A fine grain, associated with a firm meat and a velvety moist surface, is preferred. In general, coarse texture is associated with diminished tenderness (Hammond, 1940, 1942; Rambottom *et al* 1945). That toughness of meat is associated with texture, is indicated by the improvement effected by hammering meat with a rolling-pin. This has the effect of breaking down the larger muscle bundles into small ones, and decreasing the toughness.

However, the thickness of the muscle fibres comprising the bundle, considered as a separate factor from size of bundle as such, may also exert an influence. Thus, Satorius and Child (1938) found that large bundles of fine fibres are more tender than smaller bundles of thick fibres. Hammond and Appleton (1932) too, showed a tendency for the tenderest muscle to have the thinnest fibres.

In young animals, where the muscle bundles are small in size, the meat is tender. With age the muscle bundles increase in size, due to the increasing size of the component fibres, and the meat becomes tougher. Females have flesh of finer texture than males, with castrated animals occupying an intermediate position. Size of the animal too, has an effect. In small animals (rabbit, sheep) the muscle bundles are smaller and the meat is tender, whereas the meat from large animals (cattle) in which the bundles are larger is tougher. In some muscles the fibres grow much larger than others, hence bringing about an increased coarseness of their texture. For instance, *M. Gracilis* in the leg of mutton is fine-grained and tender as compared with the coarse-grained *M. Vastus lateralis* which is tough (Hammond and Appleton, 1932).

Apart from size of fibre or bundle, the distribution of the connective tissue is another factor to be considered in relation to texture. In general, the connective tissue in a fine-grained muscle is finely and evenly distributed throughout the muscle. Presumably such muscles break down easily on mastication, whereas the connective tissue may be distributed in bands and patches in coarsely grained muscle and mastication will probably be more difficult.

Methods of tenderizing meat (beating with a hammer, ripening, prolonged cooking) may all act in an incidental manner by tending to break up the grain. Either the large bundles break down into smaller ones in a mechanical fashion, or by a softening of the connective tissue holding the bundles together a similar effect may be achieved.

The difficulty is to distinguish between connective tissue content and meat texture as separate factors inducing tenderness. In the absence of measurement of texture controlled by parallel determination of connective tissue in the different muscles the relative importance of these factors is not easily decided. For instance, a higher or lower connective tissue content in a muscle may contribute to a lesser or greater degree of tenderness, quite apart from the effect of the texture characteristic of that particular muscle. In the sheep, Hammond and Appleton (1932) have shown that the extensors

and flexors of the leg and foot are more finely grained than the thigh muscles. Yet the former class are in general sinewy and tendinous, and do not constitute the meaty muscles of the leg of mutton.

5. *Changes due to Ripening of Meat.*

After death there is a chemical change in the proteins of muscle whereby the muscles become firm, but when rigor mortis passes off the coagulated muscle proteins become converted into soluble forms. Subsequently there is a swelling and softening of collagen fibres due to the lactic acid produced in the muscle after death (Moran and Smith, 1929). As may be expected, the pH of muscle in rigor is roughly proportional to its lactic acid content (Bate-Smith, 1936). According to Moran (1935) the pH of muscle falls from approximately 7.2 to 5.8. As a result of this acidification of the meat the collagen is more easily converted into soluble gelatin by the cooking process. This action is reinforced by a hydrolytic action of the ferments present in muscle, when meat is hung a long while. The latter process apparently does not play an appreciable rôle during the first thirty days (Hoagland *et al.*, 1917).

Still other possibilities are suggested by the experiment of Winkler (1939). Samples of raw pork and beef were adjusted to different pH values by injecting appropriate concentrations of lactic acid or ammonia. After storing the meat for four days at 0° C. the samples were tested for tenderness and, in general, it was found that the addition of sufficient lactic acid or ammonia makes the meat more tender. Winkler thinks it unlikely that hydrolysis of connective tissue around the muscle bundles is responsible for these observed changes in tenderness. He mentions as possible factors changes in the protein water relations, or an increased activity in protein-splitting enzymes.

Histological changes occurring in the muscle fibres of poultry during the onset and resolution of rigor mortis have been described by Hanson *et al.* (1942). First characteristic contracture nodes and internodes appear with the onset of rigor mortis. With the resolution of rigor the cell contents become thinner. Later definite breaks appear in the fibres, and finally the striated structure of the fibres changes to a granular type of structure. The authors conclude that these changes within the muscle fibres appear to be correlated with the increasing tenderness of the flesh. Similar changes were observed by Paul *et al.* (1944) in their study of beef muscles.

6. *General.*

Apart from the factors already considered it is of interest to mention certain other characters, concerning rather the individual animal.

As regards feeding, a number of investigators have shown that maintenance and sub-maintenance rations produce a peculiar rubbery consistency of the meat (Hunt, 1935; Barbella *et al.*, 1936).

As regards heredity, it is likely that breeding may play an important part in producing differences in quality of meat. For instance, the progeny of one sire were shown to have meat of considerably greater toughness than that of another sire (Report B.A.I. 1941). Thus a superior breeding value is established as having material influence on meat quality, apart from factors such as age of animals, their feeding and management, the handling and storing of carcasses (Report B.A.I., 1937, 1939). This fact, that some animals possess a natural tendency to tender meat which is lacking in others, is also brought out by Warner and Alexander (1932).

SECTION 4.—METHODS TO INCREASE THE TENDERNESS OF MEAT.

1. *Hanging.*

It is accepted that hanging meat for a period after slaughter increases its palatability. This is mainly due to the marked increase in tenderness but, in addition, the meat is also more juicy and richer in flavour. Moran and Smith (1929) pointed out that the British public did not appreciate this improved palatability, and mentioned the inertia of the trade to introduce this reform. Consequently, in Britain, carcasses were generally allowed to hang for only twenty-four hours after slaughter to cool and set, before removal to the shops for sale. Moran and Smith report an increase in tenderness of beef of ten per cent. after hanging seven days at 41° F., increasing to thirty-one per cent. after seventeen days. They recommend hanging sides or quarters for ten to twelve days at 36 to 38° F., after an initial cooling of the carcass for one to two days at 31 to 33° F.

In the United States ripening of meat is widely practised. Consumers appreciate the fact that hanging meat increases its tenderness and improves its flavour. As a result a considerable amount of beef is held in cold storage for two to six weeks before delivery to the retail trade. However, it will be noted that the process of ageing can be satisfactorily applied only to carcasses with a good covering of fat. With poorly finished beef the ripening period must be short, due to the increased susceptibility to microbial spoilage, greater shrinkage, etc. Mainly ribs, loins, and hindquarters of high-grade well-fattened cattle are kept at about 36° F. in an atmosphere of fairly low relative humidity. Pork consumers prefer unripened meat. While there is no definite choice in the lamb trade, some customers ask for ripening of hindquarters. In general, however, lamb is moved into the retail trade as quickly as possible to reduce shrinkage losses during storage.

In beef, Hiner and Hankins (1941) found that in order to keep deterioration down to a minimum and yet obtain a large tenderizing effect it is advisable to hang cuts from Low to Good grade beef carcasses for not more than fifteen days at 34° F. The total tenderizing effect between the fifth and thirty-fifth day was 28.2 per cent., of which twenty per cent. occurred from five to fifteen days, and only eight per cent. from fifteen to thirty-five days.

Warner and Alexander (1932) investigated the changes in ripened legs of lamb. Tenderness increased during the first ten days of storage, but during the next ten days only a slight increase was apparent.

Although prolonged ripening is an expensive process, consumers in the United States consider it is justified by the increased palatability, especially tenderness. The disadvantages associated with ripening are mentioned by Ewell (1940). They are, the cost of refrigeration (average five per cent.); the interest on capital invested in meat; the loss of weight (two to ten per cent.); an impaired colour or bloom; and the necessity for trimming as a result of spoilage due to bacteria and moulds.

2. *The Tenderay Process.*

In order to overcome the disadvantages associated with hanging meat a higher temperature may be used to increase the speed of ripening. Steiner (1939), Ewell (1940) showed that the ripening effect is increased as the temperature is raised from 32 to 60° F. As the temperature is increased the rate of tenderizing rises, so that the time required for a given degree of tendering

is greatly reduced, e.g., one to three days at 60° F. is equivalent to twenty-one days or longer at 34 to 37° F. However, as the temperature is raised spoilage (bacterial, mould, rancidity) increases even more rapidly than the speed of tendering. Furthermore at high temperatures the bloom is badly spoilt, unless the relative humidity is high, which again favours bacterial and mould spoilage.

An advance became possible when Harvey Rentschler showed that ultra-violet radiation can be used to retard spoilage and reduce shrinkage, when ripening is carried out at a high temperature and high relative humidity. Due to retarded bacterial and mould development the meat can be held at warmer temperatures, and the ripening process is accelerated. Although the cost of installing and operating "Sterilamps" is slight, considerable economic benefit results from the shortened period of storage and a quicker turnover.

Ewell (1939) describes the advantages of this system of ripening. A very slight coagulation occurs on the meat surface, too slight to affect appreciably its appearance or taste. At the same time this coagulation is sufficient to reduce shrinkage materially (several per cent.), this being in addition to the reduction in evaporation loss made possible by the maintenance of a relative humidity of about ninety per cent. Without the "Sterilamp" such humidities result in serious loss after only a few days, due to a surface growth of bacteria and the consequent necessity for trimming.

Ewell (1941) enquired into the factors making possible these desirable effects. Apart from the direct radiation inhibiting or killing air-borne organisms and, to a lesser extent, micro-organisms on the meat surface, a minute concentration of ozone ($\frac{1}{1000}$ p.p.m.) is produced in the radiated air.

With a properly controlled air circulation this ozone reaches meat surfaces shaded from the direct radiation and limits development of organisms on surfaces not directly exposed to "Sterilamps". Apparently these two agents acting in conjunction bring about the favourable effect.

Moulton (1939) describes how beef is aged, at 55 to 58° F. and ninety per cent. relative humidity, in three to four days by the Sterilamp process as compared with several weeks under the methods previously employed. Griswold and Wharton (1941) found that meat held for forty-eight hours at 60° F. under continuous irradiation is slightly more tender than that held at 36° F. for the same length of time. Ensminger *et al* (1942) report that the use of ultra-violet light reduced the surface microflora of beef, but at the same time a greasy appearance was imparted to the tallow during ageing periods of seven to fourteen days.

Deane (1942) mentions the possible ill-effects to personnel of exposure to the Sterilamps, e.g., the radiation arousing latent tuberculosis, conjunctivitis resulting from direct exposure of the eyes to ultra-violet lighting. People are warned not to look directly at the lamps even momentarily, and protection for the eyes is provided by means of a cap with a visor.

The relation of ultra-violet radiation, and temperature during ageing, to quality in beef is reviewed by McIntosh *et al* (1942). They found that ultra-violet radiation reduces the surface microflora of beef. They could detect no difference in texture and tenderness of good quality beef shortloins

aged for seven and fourteen days, with and without ultra-violet light at 34° and 50° F. However, they state that the meat was of such good quality initially that it would be difficult to effect improvement. They cite research at the Mellon Institute which showed that the general effect of using high humidity, higher temperature, and Sterilamps was to advance beef at least one government grade.

Porter (1940) quotes results at one market producing a large quantity of tenderay beef. The trimming losses were reduced from approximately six per cent. to less than half of one per cent., and shrinkage to an equally negligible figure, as compared with the older method of hanging beef.

3. *The Birdseye Process of Quick-freezing.*

Before considering the effect of freezing on the tenderness of meat it is desirable to consider the phenomenal development of the Birdseye process. By this process cuts of meat are prepared, wrapped, and placed in a carton for freezing upon a moving belt. At the entrance to the freezing tunnel an overhead belt is kept gently and uniformly pressed against the package to ensure a very rapid heat transfer. As both belts are cooled to very low temperatures, by sprays of calcium chloride brine cooled to about 50° F. below freezing point, the material is frozen rapidly at an extremely low temperature. By virtue of the speed of freezing a great improvement is effected over other freezing processes, as the original structure of the meat is retained.

The possibilities of this method aroused the interest of packers in the United States. As a result quick-freezing was applied in the meat-packing industry in connection with cuts of meat (fillet, steak, roasts), and the commercial distribution of quick-frozen meat to retail trades started about 1930. The advantage of the handy package may be judged from the fact that Kolbe (1930) estimates that in the retail trade economies of 2.5 cents per pound are made through handling the packed products. Another advantage is that the packer retains all waste fractions (fat, bone, trimmings), which can be usefully employed.

In addition to quick-freezing, another development is the freezer locker branch of the industry. Originally started in the middle 1920's this has made enormous progress all over the United States since 1937. In 1941 about 4,000 freezer locker plants were in operation, increasing at the rate of about 100 per month (Carlton, 1941). Assuming an average of 200 rented lockers per plant, the 4,000 lockers were serving upwards of 800,000 families, or a turnover of about 480,000,000 pounds per year of which meats comprised about seventy-five per cent. of the locker output.

Space for the proper storage of quick-frozen foods is provided by these freezing lockers, and commercial distributors are also enabled to serve remote districts. Included in the service provided by the locker plant is also the purchase of carcasses wholesale from the packers for patrons, and the deboning, cutting, wrapping and labelling of the separate cuts of meat. At wholesale prices meats are generally ten to twelve cents per pound cheaper than over the counter retail prices. Thus it is estimated that the average family using 800 pounds of meat per year saves about \$60 annually, after deducting the cost of processing and the locker rental.

Little was known of the effect of freezing on tenderness of meat, until the use of temperatures far below freezing point became customary. Then

Tressler and Murray (1932) compared chilled and quick-frozen beef and reported the process of quick-freezing had made the beef more tender. In one experiment a quick-frozen grade C steak became as tender as a non-frozen grade A steak (Tressler *et al.*, 1932). Hankins and Hiner (1938, 1940) showed that all freezing temperatures studied (20° , -10° , -40° F.) made meat more tender than the unfrozen controls. As there was no real difference in tenderizing steaks between -10° F. and -40° F. they recommend the former temperature as being economical and practical for this purpose. In poultry too, the process of quick-freezing renders the thigh muscles consistently more tender than the corresponding muscles from unfrozen control birds (Stewart *et al.*, 1945).

Studies were also undertaken to determine the period of time beef should age before it is frozen, in order to achieve a maximum tenderizing effect (Hankins and Hiner, 1941; Hiner and Hankins, 1941). These workers confirmed their previous finding that quick-freezing increases tenderness of beef. By comparison with beef aged thirty-five days at 34° F., beef was of similar tenderness when frozen at -10° F. after ageing it for only five days at 34° F. The authors recommend that cuts from low good grade beef carcasses should be aged not more than fifteen days at 34° F., then frozen at -10° F. Bray *et al.* (1942), who also studied the effect of freezing aged beef, conclude that after beef has been ripened freezing does not increase its tenderness still further.

All workers do not agree regarding the tenderizing effect produced by freezing meat. Thus, Bull *et al.* (1937) reported that the flavour and quality of quick-frozen meats and those not frozen are similar, except that frozen pork is consistently more tender and juicy than the fresh. Paul and Child (1937) too, found no significant difference in tenderness of unfrozen beef and beef frozen at 0.6° F. However, Brady *et al.* (1942) point out that under the conditions of this experiment it took twenty-five hours for the interior of the meat to reach this temperature, whereas in their own experiment an average of only seven hours was required for the interior of the quick-frozen meat to reach a temperature of 0° F. Thus, not only room temperature at which freezing takes place, but also the actual freezing rate plays an important part in the tenderizing process. Owing to differences in the mechanical set-up cuts of meat may be frozen in markedly different lengths of time, even under the same temperature conditions.

Recent work by Hiner, Madsen and Hankins (1945) throws light on the nature of the histological changes occurring during the quick-freezing of meat. As these authors review the literature dealing with the histology of ice-formation in frozen meat, no mention is made here of the earlier work in this field. In their study, beef shearing tests showed a consistent increase in tenderness as the freezing temperature is progressively lowered from 18° F. to -114° . Histological sections of the frozen beef showed that *interfibrillar* ice is formed at relatively high freezing temperatures (18° F.). As the freezing temperature is lowered (0° , -10° , -40° , -114°), *intrafibrillar* freezing becomes progressively more extensive. The expansion occurring when the cell moisture is frozen *intracellularly* tends to rupture the muscle fibre itself, and at -114° F. nearly every fibre is ruptured. Hiner and his co-workers believe that the tenderizing effect of low freezing temperatures is largely due to disintegration of fibres, resulting from *intrafibrillar* ice formation, but partly also by stretching and rupture of the interstitial connective tissue.

With the impetus afforded by war conditions another major development has been brought about. Quick-freezing can now be applied to whole quarters of beef. Previously only small cuts could be treated, but by the Brewster process whole quarters of beef are boned out and frozen in the same number of hours as it formerly took days (Brewster, 1944). Stated briefly, warm meat is deboned immediately after killing and the deboned beef is compressed into moulds, for freezing by brine sprays at -14.8°F. , for six hours. After freezing, the moulds are thawed in approximately forty seconds sufficiently for the block of frozen meat to be removed from the mould, for bagging and shipment in freezers at -18°F. The chief merit of the new process is the saving of roughly thirty-five per cent. of storage and transport space as compared with *ordinary type boneless beef*. The possibilities of this new development are enhanced by the fact that this quick-frozen beef is claimed to be perhaps even better in palatability than pre-war *chilled beef*. Brewster suggests this is because freezing is carried out before rigor mortis sets in. His observations show that meat which is "quickly frozen immediately after killing retains during its frozen state, and on thawing out, the characteristics of freshly-killed meat". Bate-Smith (1944) discusses the theoretical basis making possible this desirable state of affairs.

4. *The use of Enzymes for Tenderizing Meat.*

Yet another development has been the utilization of enzymes in tenderizing meat. For instance, 223,000 pounds of crude papain were imported into the United States in 1938 in comparison with 54,000 pounds in 1932, its greatest use being in the manufacture of meat tenderisers (Ramsbottom and Rinehart, 1940).

These authors also mention the first industrial use of bromelin (the proteolytic enzyme in pineapple juice) in the meat-packing industry. Here the casing containing the sausage meat is treated by spraying bromelin as a fine mist onto the casing of large "frankfurters" and sausages. By virtue of the action of the bromelin the complex natural proteins of the animal casings are broken-down into proteins of simpler composition less resistant to mastication and digestion. Thus penetrometer test readings, of treated as compared with untreated "Frankfurters," yielded average readings of 75.7 and 122.2 units.

5. *Cookery.*

In the preceding pages an attempt has been made to discuss briefly the general problem of toughness of meat, and the methods whereby a degree of tenderizing may be effected. Cookery has not been mentioned. Obviously, this final stage is extremely important, as indifferent preparation may offset the benefits of skilled animal husbandry and meat processing up to this point. It is clear that cooking should be such that the quality and flavour of good meat are in no way impaired. Furthermore, skill must also be exercised to improve the palatability of inferior quality meat. That workers are alive to the importance of the cookery process is evidenced by the vast amount of information available regarding this aspect of the problem. American workers, mainly, have pioneered the investigation of scientific cookery. Enormous progress has been reported in the United States since 1925, when co-operative meat investigations were first undertaken (U.S. Conf. Co-op. Meat Investigations, 1937, 1942).

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CHAPTER VII—APPENDIX.

TABLE A.
Analysis of Measurements of Fiber Diameter Right Gastrocnemius Muscle, Rabbit No. 16
(True section Method [Diameter calculated from Mean of Horizontal and Vertical Dimensions of Fiber])

NUMBER OF FIBERS COMPRISING EACH SELECTION

No.	Mean Fiber Diameter (microns) ± S. D.	25	30	35	40	50	55	60	65	70	75	80	85	90	100	105	110	115	120	125	130	135	140	145	150	155	160	165	170	175	180	185	190	195	200	205	210	215	220	225	230	235	240	245	250	255	260	265	270	275	280	285	290	295	300	305	310	315	320	325	330	335	340	345	350	355	360	365	370	375	380	385	390	395	400	405	410	415	420	425	430	435	440	445	450	455	460	465	470	475	480	485	490	495	500	505	510	515	520	525	530	535	540	545	550	555	560	565	570	575	580	585	590	595	600	605	610	615	620	625	630	635	640	645	650	655	660	665	670	675	680	685	690	695	700	705	710	715	720	725	730	735	740	745	750	755	760	765	770	775	780	785	790	795	800	805	810	815	820	825	830	835	840	845	850	855	860	865	870	875	880	885	890	895	900	905	910	915	920	925	930	935	940	945	950	955	960	965	970	975	980	985	990	995	1000																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
586	Mean Fiber Diameter (microns) ± S. D.	18.97	19.06	19.23	19.31	19.45	19.55	19.65	19.75	19.85	19.95	20.05	20.15	20.25	20.35	20.45	20.55	20.65	20.75	20.85	20.95	21.05	21.15	21.25	21.35	21.45	21.55	21.65	21.75	21.85	21.95	22.05	22.15	22.25	22.35	22.45	22.55	22.65	22.75	22.85	22.95	23.05	23.15	23.25	23.35	23.45	23.55	23.65	23.75	23.85	23.95	24.05	24.15	24.25	24.35	24.45	24.55	24.65	24.75	24.85	24.95	25.05	25.15	25.25	25.35	25.45	25.55	25.65	25.75	25.85	25.95	26.05	26.15	26.25	26.35	26.45	26.55	26.65	26.75	26.85	26.95	27.05	27.15	27.25	27.35	27.45	27.55	27.65	27.75	27.85	27.95	28.05	28.15	28.25	28.35	28.45	28.55	28.65	28.75	28.85	28.95	29.05	29.15	29.25	29.35	29.45	29.55	29.65	29.75	29.85	29.95	30.05	30.15	30.25	30.35	30.45	30.55	30.65	30.75	30.85	30.95	31.05	31.15	31.25	31.35	31.45	31.55	31.65	31.75	31.85	31.95	32.05	32.15	32.25	32.35	32.45	32.55	32.65	32.75	32.85	32.95	33.05	33.15	33.25	33.35	33.45	33.55	33.65	33.75	33.85	33.95	34.05	34.15	34.25	34.35	34.45	34.55	34.65	34.75	34.85	34.95	35.05	35.15	35.25	35.35	35.45	35.55	35.65	35.75	35.85	35.95	36.05	36.15	36.25	36.35	36.45	36.55	36.65	36.75	36.85	36.95	37.05	37.15	37.25	37.35	37.45	37.55	37.65	37.75	37.85	37.95	38.05	38.15	38.25	38.35	38.45	38.55	38.65	38.75	38.85	38.95	39.05	39.15	39.25	39.35	39.45	39.55	39.65	39.75	39.85	39.95	40.05	40.15	40.25	40.35	40.45	40.55	40.65	40.75	40.85	40.95	41.05	41.15	41.25	41.35	41.45	41.55	41.65	41.75	41.85	41.95	42.05	42.15	42.25	42.35	42.45	42.55	42.65	42.75	42.85	42.95	43.05	43.15	43.25	43.35	43.45	43.55	43.65	43.75	43.85	43.95	44.05	44.15	44.25	44.35	44.45	44.55	44.65	44.75	44.85	44.95	45.05	45.15	45.25	45.35	45.45	45.55	45.65	45.75	45.85	45.95	46.05	46.15	46.25	46.35	46.45	46.55	46.65	46.75	46.85	46.95	47.05	47.15	47.25	47.35	47.45	47.55	47.65	47.75	47.85	47.95	48.05	48.15	48.25	48.35	48.45	48.55	48.65	48.75	48.85	48.95	49.05	49.15	49.25	49.35	49.45	49.55	49.65	49.75	49.85	49.95	50.05	50.15	50.25	50.35	50.45	50.55	50.65	50.75	50.85	50.95	51.05	51.15	51.25	51.35	51.45	51.55	51.65	51.75	51.85	51.95	52.05	52.15	52.25	52.35	52.45	52.55	52.65	52.75	52.85	52.95	53.05	53.15	53.25	53.35	53.45	53.55	53.65	53.75	53.85	53.95	54.05	54.15	54.25	54.35	54.45	54.55	54.65	54.75	54.85	54.95	55.05	55.15	55.25	55.35	55.45	55.55	55.65	55.75	55.85	55.95	56.05	56.15	56.25	56.35	56.45	56.55	56.65	56.75	56.85	56.95	57.05	57.15	57.25	57.35	57.45	57.55	57.65	57.75	57.85	57.95	58.05	58.15	58.25	58.35	58.45	58.55	58.65	58.75	58.85	58.95	59.05	59.15	59.25	59.35	59.45	59.55	59.65	59.75	59.85	59.95	60.05	60.15	60.25	60.35	60.45	60.55	60.65	60.75	60.85	60.95	61.05	61.15	61.25	61.35	61.45	61.55	61.65	61.75	61.85	61.95	62.05	62.15	62.25	62.35	62.45	62.55	62.65	62.75	62.85	62.95	63.05	63.15	63.25	63.35	63.45	63.55	63.65	63.75	63.85	63.95	64.05	64.15	64.25	64.35	64.45	64.55	64.65	64.75	64.85	64.95	65.05	65.15	65.25	65.35	65.45	65.55	65.65	65.75	65.85	65.95	66.05	66.15	66.25	66.35	66.45	66.55	66.65	66.75	66.85	66.95	67.05	67.15	67.25	67.35	67.45	67.55	67.65	67.75	67.85	67.95	68.05	68.15	68.25	68.35	68.45	68.55	68.65	68.75	68.85	68.95	69.05	69.15	69.25	69.35	69.45	69.55	69.65	69.75	69.85	69.95	70.05	70.15	70.25	70.35	70.45	70.55	70.65	70.75	70.85	70.95	71.05	71.15	71.25	71.35	71.45	71.55	71.65	71.75	71.85	71.95	72.05	72.15	72.25	72.35	72.45	72.55	72.65	72.75	72.85	72.95	73.05	73.15	73.25	73.35	73.45	73.55	73.65	73.75	73.85	73.95	74.05	74.15	74.25	74.35	74.45	74.55	74.65	74.75	74.85	74.95	75.05	75.15	75.25	75.35	75.45	75.55	75.65	75.75	75.85	75.95	76.05	76.15	76.25	76.35	76.45	76.55	76.65	76.75	76.85	76.95	77.05	77.15	77.25	77.35	77.45	77.55	77.65	77.75	77.85	77.95	78.05	78.15	78.25	78.35	78.45	78.55	78.65	78.75	78.85	78.95	79.05	79.15	79.25	79.35	79.45	79.55	79.65	79.75	79.85	79.95	80.05	80.15	80.25	80.35	80.45	80.55	80.65	80.75	80.85	80.95	81.05	81.15	81.25	81.35	81.45	81.55	81.65	81.75	81.85	81.95	82.05	82.15	82.25	82.35	82.45	82.55	82.65	82.75	82.85	82.95	83.05	83.15	83.25	83.35	83.45	83.55	83.65	83.75	83.85	83.95	84.05	84.15	84.25	84.35	84.45	84.55	84.65	84.75	84.85	84.95	85.05	85.15	85.25	85.35	85.45	85.55	85.65	85.75	85.85	85.95	86.05	86.15	86.25	86.35	86.45	86.55	86.65	86.75	86.85	86.95	87.05	87.15	87.25	87.35	87.45	87.55	87.65	87.75	87.85	87.95	88.05	88.15	88.25	88.35	88.45	88.55	88.65	88.75	88.85	88.95	89.05	89.15	89.25	89.35	89.45	89.55	89.65	89.75	89.85	89.95	90.05	90.15	90.25	90.35	90.45	90.55	90.65	90.75	90.85	90.95	91.05	91.15	91.25	91.35	91.45	91.55	91.65	91.75	91.85	91.95	92.05	92.15	92.25	92.35	92.45	92.55	92.65	92.75	92.85	92.95	93.05	93.15	93.25	93.35	93.45	93.55	93.65	93.75	93.85	93.95	94.05	94.15	94.25	94.35	94.45	94.55	94.65	94.75	94.85	94.95	95.05	95.15	95.25	95.35	95.45	95.55	95.65	95.75	95.85	95.95	96.05	96.15	96.25	96.35	96.45	96.55	96.65	96.75	96.85	96.95	97.05	97.15	97.25	97.35	97.45	97.55	97.65	97.75	97.85	97.95	98.05	98.15	98.25	98.35	98.45	98.55	98.65	98.75	98.85	98.95	99.05	99.15	99.25	99.35	99.45	99.55	99.65	99.75	99.85	99.95	100.05	100.15	100.25	100.35	100.45	100.55	100.65	100.75	100.85	100.95	101.05	101.15	101.25	101.35	101.45	101.55	101.65	101.75	101.85	101.95	102.05	102.15	102.25	102.35	102.45	102.55	102.65	102.75	102.85	102.95	103.05	103.15	103.25	103.35	103.45	103.55	103.65	103.75	103.85	103.95	104.05	104.15	104.25	104.35	104.45	104.55	104.65	104.75	104.85	104.95	105.05	105.15	105.25	105.35	105.45	105.55	105.65	105.75	105.85	105.95	106.05	106.15	106.25	106.35	106.45	106.55	106.65	106.75	106.85	106.95	107.05	107.15	107.25	107.35	107.45	107.55	107.65	107.75	107.85	107.95	108.05	108.15	108.25	108.35	108.45	108.55	108.65	108.75	108.85	108.95	109.05	109.15	109.25	109.35	109.45	109.55	109.65	109.75	109.85	109.95	110.05	110.15	110.25	110.35	110.45	110.55	110.65	110.75	110.85	110.95	111.05	111.15	111.25	111.35	111.45	111.55	111.65	111.75	111.85	111.95	112.05	112.15	112.25	112.35	112.45	112.55	112.65	112.75	112.85	112.95	113.05	113.15	113.25	113.35	113.45	113.55	113.65	113.75	113.85	113.95	114.05	114.15	114.25	114.35	114.45	114.55	114.65	114.75	114.85	114.95	115.05	115.15	115.25	115.35	115.45	115.55	115.65	115.75	115.85	115.95	116.05	116.15	116.25	116.35	116.45	116.55	116.65	116.75	116.85	116.95	117.05	117.15	117.25	117.35	117.45	117.55	117.65	117.75	117.85	117.95	118.05	118.15	118.25	118.35	118.45	118.55	118.65	118.75	118.85	118.95	119.05	119.15	119.25	119.35	119.45	119.55	119.65	119.75	119.85	119.95	120.05	120.15	120.25	120.35	120.45	120.55	120.65	120.75	120.85	120.95	121.05	121.15	121.25	121.35	121.45	121.55	121.65	121.75	121.85	121.95	122.05	122.15	122.25	122.35	122.45	122.

TABLE B.
Analysis of Measurements of Fibre Diameter. Right Gastrocnemius Muscle. Rabbit No. 16.
Hemmon's Method (Diameter measured directly across short lengths of fibre).

NUMBER OF FIGURES COMBINING EACH SELECTION.

[illegible]

TABLE C.
Analysis of Measurements of Pigeon Diameter, Right Pigeon Muscle, Rabbit No. 10.
Cross-Section method (Standard calculated from Means of Horizontal and Vertical Dimensions of Fiber).

NUMBER OF FIBERS COMPRISING EACH SECTION.

	50	125	150	175	200	225	250	275	300	325	350	375	400	425	450	475	500
Mean Fiber Diameter (Lanometer Scale) - 200	17.98	18.05	18.14	18.26	18.38	18.50	18.62	18.74	18.86	18.98	19.10	19.22	19.34	19.46	19.58	19.70	19.82
Standard Deviation	0.008	0.009	0.010	0.011	0.012	0.013	0.014	0.015	0.016	0.017	0.018	0.019	0.020	0.021	0.022	0.023	0.024
Coefficient of Variability	0.045%	0.050%	0.055%	0.061%	0.065%	0.070%	0.075%	0.080%	0.085%	0.090%	0.095%	0.100%	0.105%	0.110%	0.115%	0.120%	0.125%
Mean Fiber Diameter (Lanometer Scale) - 200	18.37	18.10	18.20	18.31	18.42	18.53	18.64	18.75	18.86	18.97	19.08	19.19	19.30	19.41	19.52	19.63	19.74
Standard Deviation	0.009	0.010	0.011	0.012	0.013	0.014	0.015	0.016	0.017	0.018	0.019	0.020	0.021	0.022	0.023	0.024	0.025
Coefficient of Variability	0.049%	0.056%	0.061%	0.066%	0.071%	0.076%	0.081%	0.086%	0.091%	0.096%	0.101%	0.106%	0.111%	0.116%	0.121%	0.126%	0.131%
Mean Fiber Diameter (Lanometer Scale) - 200	18.08	18.15	18.22	18.30	18.38	18.46	18.54	18.62	18.70	18.78	18.86	18.94	19.02	19.10	19.18	19.26	19.34
Standard Deviation	0.008	0.009	0.010	0.011	0.012	0.013	0.014	0.015	0.016	0.017	0.018	0.019	0.020	0.021	0.022	0.023	0.024
Coefficient of Variability	0.044%	0.050%	0.055%	0.061%	0.065%	0.070%	0.075%	0.080%	0.085%	0.090%	0.095%	0.100%	0.105%	0.110%	0.115%	0.120%	0.125%
Mean Fiber Diameter (Lanometer Scale) - 200	18.08	18.15	18.22	18.30	18.38	18.46	18.54	18.62	18.70	18.78	18.86	18.94	19.02	19.10	19.18	19.26	19.34
Standard Deviation	0.008	0.009	0.010	0.011	0.012	0.013	0.014	0.015	0.016	0.017	0.018	0.019	0.020	0.021	0.022	0.023	0.024
Coefficient of Variability	0.044%	0.050%	0.055%	0.061%	0.065%	0.070%	0.075%	0.080%	0.085%	0.090%	0.095%	0.100%	0.105%	0.110%	0.115%	0.120%	0.125%
Mean Fiber Diameter (Lanometer Scale) - 200	18.08	18.15	18.22	18.30	18.38	18.46	18.54	18.62	18.70	18.78	18.86	18.94	19.02	19.10	19.18	19.26	19.34
Standard Deviation	0.008	0.009	0.010	0.011	0.012	0.013	0.014	0.015	0.016	0.017	0.018	0.019	0.020	0.021	0.022	0.023	0.024
Coefficient of Variability	0.044%	0.050%	0.055%	0.061%	0.065%	0.070%	0.075%	0.080%	0.085%	0.090%	0.095%	0.100%	0.105%	0.110%	0.115%	0.120%	0.125%
Mean Fiber Diameter (Lanometer Scale) - 200	18.08	18.15	18.22	18.30	18.38	18.46	18.54	18.62	18.70	18.78	18.86	18.94	19.02	19.10	19.18	19.26	19.34
Standard Deviation	0.008	0.009	0.010	0.011	0.012	0.013	0.014	0.015	0.016	0.017	0.018	0.019	0.020	0.021	0.022	0.023	0.024
Coefficient of Variability	0.044%	0.050%	0.055%	0.061%	0.065%	0.070%	0.075%	0.080%	0.085%	0.090%	0.095%	0.100%	0.105%	0.110%	0.115%	0.120%	0.125%
Mean Fiber Diameter (Lanometer Scale) - 200	18.08	18.15	18.22	18.30	18.38	18.46	18.54	18.62	18.70	18.78	18.86	18.94	19.02	19.10	19.18	19.26	19.34
Standard Deviation	0.008	0.009	0.010	0.011	0.012	0.013	0.014	0.015	0.016	0.017	0.018	0.019	0.020	0.021	0.022	0.023	0.024
Coefficient of Variability	0.044%	0.050%	0.055%	0.061%	0.065%	0.070%	0.075%	0.080%	0.085%	0.090%	0.095%	0.100%	0.105%	0.110%	0.115%	0.120%	0.125%

NUMBER OF YEARS CONTAINING EACH SILENT

[illegible]

M. PROAH M.AJOR:

Sexual Number of Individual	Age in Days	Weight in Grams	Dimensions.										Dimensions.											
			Wing in Centimeters	Width at A in Centimeters	Width at B in Centimeters	Width at D in Centimeters	Width at E in Centimeters	Depth at A in Centimeters	Depth at B in Centimeters	Depth at E in Centimeters	Length in Centimeters	Width at A in Centimeters	Width at C in Centimeters	Width at D in Centimeters	Width at E in Centimeters	Depth at A in Centimeters	Depth at B in Centimeters	Depth at E in Centimeters						
100 Day Group	74	0.030	1.0	0.21	0.34	0.35	0.25	0.24	0.10	0.18	0.17	0.12	0.12	0.07	0.52	0.43	0.45	0.30	0.30	0.32	0.04	0.07	0.08	0.11
	75	0.035	0.9	0.20	0.34	0.34	0.27	0.30	0.12	0.14	0.16	0.13	0.08	0.05	0.50	0.37	0.38	0.40	0.36	0.52	0.04	0.06	0.08	0.11
	77	0.045	0.9	0.20	0.34	0.34	0.27	0.30	0.12	0.14	0.16	0.13	0.08	0.05	0.50	0.37	0.38	0.40	0.36	0.52	0.04	0.06	0.08	0.11
	81	0.054	1.1	0.28	0.35	0.37	0.34	0.31	0.11	0.11	0.12	0.13	0.08	0.05	0.51	0.30	0.30	0.36	0.37	0.54	0.04	0.07	0.07	0.10
	86	0.061	1.0	0.29	0.36	0.37	0.34	0.31	0.11	0.11	0.12	0.13	0.08	0.05	0.51	0.30	0.30	0.36	0.37	0.54	0.04	0.07	0.07	0.10
	97	0.071	1.0	0.29	0.36	0.37	0.34	0.31	0.11	0.11	0.12	0.13	0.08	0.05	0.51	0.30	0.30	0.36	0.37	0.54	0.04	0.07	0.07	0.10
	101	0.082	0.9	0.24	0.30	0.36	0.42	0.40	0.22	0.10	0.10	0.12	0.08	0.05	0.50	0.37	0.38	0.40	0.36	0.52	0.04	0.07	0.08	0.11
	104	0.086	1.0	0.24	0.30	0.36	0.42	0.40	0.22	0.10	0.10	0.12	0.08	0.05	0.50	0.37	0.38	0.40	0.36	0.52	0.04	0.07	0.08	0.11
	105	0.084	1.0	0.25	0.30	0.36	0.42	0.40	0.22	0.10	0.10	0.12	0.08	0.05	0.50	0.37	0.38	0.40	0.36	0.52	0.04	0.07	0.08	0.11
	Mean	0.054	1.2	0.24	0.31	0.35	0.35	0.32	0.26	0.09	0.09	0.11	0.09	0.08	0.06	0.52	0.39	0.41	0.42	0.37	0.53	0.04	0.07	0.08
150 Day Group	102	0.092	1.0	0.35	0.44	0.45	0.37	0.35	0.15	0.17	0.17	0.15	0.10	0.06	0.61	0.50	0.46	0.41	0.46	0.62	0.04	0.07	0.08	0.10
	104	0.095	1.0	0.37	0.47	0.43	0.38	0.35	0.13	0.14	0.14	0.13	0.10	0.06	0.60	0.51	0.47	0.44	0.47	0.62	0.04	0.07	0.08	0.10
	105	0.093	1.2	0.36	0.35	0.53	0.50	0.37	0.12	0.15	0.11	0.10	0.06	0.56	0.54	0.41	0.47	0.44	0.44	0.57	0.04	0.06	0.07	0.09
	106	0.093	1.2	0.36	0.35	0.53	0.50	0.37	0.12	0.15	0.11	0.10	0.06	0.56	0.54	0.41	0.47	0.44	0.44	0.57	0.04	0.06	0.07	0.09
	107	0.091	1.0	0.36	0.36	0.50	0.50	0.34	0.13	0.13	0.14	0.12	0.08	0.58	0.58	0.43	0.46	0.46	0.46	0.57	0.04	0.06	0.07	0.09
	108	0.091	1.2	0.36	0.36	0.50	0.50	0.34	0.13	0.13	0.14	0.12	0.08	0.58	0.58	0.43	0.46	0.46	0.46	0.57	0.04	0.06	0.07	0.09
	109	0.091	1.2	0.36	0.36	0.50	0.50	0.34	0.13	0.13	0.14	0.12	0.08	0.58	0.58	0.43	0.46	0.46	0.46	0.57	0.04	0.06	0.07	0.09
	110	0.092	1.2	0.30	0.40	0.47	0.43	0.38	0.14	0.16	0.15	0.13	0.10	0.06	0.59	0.52	0.58	0.58	0.58	0.62	0.04	0.06	0.07	0.09
	111	0.091	1.0	0.31	0.41	0.54	0.50	0.40	0.13	0.13	0.13	0.13	0.10	0.06	0.59	0.52	0.58	0.58	0.58	0.62	0.04	0.06	0.07	0.09
	Mean	0.092	1.1	0.31	0.40	0.47	0.43	0.38	0.14	0.16	0.15	0.13	0.10	0.06	0.59	0.52	0.58	0.58	0.58	0.62	0.04	0.06	0.07	0.09
225 Day Group	124	0.2019	1.7	0.46	0.54	0.62	0.55	0.36	0.22	0.25	0.23	0.15	0.09	0.20	0.55	0.50	0.49	0.06	0.02	0.04	0.07	0.10	0.12	0.14
	125	0.186	1.7	0.46	0.54	0.62	0.55	0.36	0.22	0.25	0.23	0.15	0.09	0.20	0.55	0.50	0.49	0.06	0.02	0.04	0.07	0.10	0.12	0.14
	133	0.180	2.0	0.54	0.55	0.64	0.58	0.40	0.23	0.12	0.16	0.18	0.08	0.49	0.43	0.44	0.07	0.02	0.04	0.07	0.10	0.12	0.14	
	134	0.180	2.0	0.54	0.55	0.64	0.58	0.40	0.23	0.12	0.16	0.18	0.08	0.49	0.43	0.44	0.07	0.02	0.04	0.07	0.10	0.12	0.14	
	135	0.180	2.0	0.54	0.55	0.64	0.58	0.40	0.23	0.12	0.16	0.18	0.08	0.49	0.43	0.44	0.07	0.02	0.04	0.07	0.10	0.12	0.14	
	136	0.180	2.0	0.54	0.55	0.64	0.58	0.40	0.23	0.12	0.16	0.18	0.08	0.49	0.43	0.44	0.07	0.02	0.04	0.07	0.10	0.12	0.14	
	137	0.180	2.0	0.54	0.55	0.64	0.58	0.40	0.23	0.12	0.16	0.18	0.08	0.49	0.43	0.44	0.07	0.02	0.04	0.07	0.10	0.12	0.14	
	138	0.180	2.0	0.54	0.55	0.64	0.58	0.40	0.23	0.12	0.16	0.18	0.08	0.49	0.43	0.44	0.07	0.02	0.04	0.07	0.10	0.12	0.14	
	139	0.180	2.0	0.54	0.55	0.64	0.58	0.40	0.23	0.12	0.16	0.18	0.08	0.49	0.43	0.44	0.07	0.02	0.04	0.07	0.10	0.12	0.14	
	Mean	0.180	2.0	0.54	0.55	0.64	0.58	0.40	0.23	0.12	0.16	0.18	0.08	0.49	0.43	0.44	0.07	0.02	0.04	0.07	0.10	0.12	0.14	0.14
300 Day Group	141	0.240	2.4	0.54	0.64	0.72	0.64	0.43	0.18	0.20	0.20	0.15	0.09	0.20	0.55	0.50	0.49	0.06	0.02	0.04	0.07	0.10	0.12	0.14
	142	0.240	2.4	0.54	0.64	0.72	0.64	0.43	0.18	0.20	0.20	0.15	0.09	0.20	0.55	0.50	0.49	0.06	0.02	0.04	0.07	0.10	0.12	0.14
	143	0.240	2.4	0.54	0.64	0.72	0.64	0.43	0.18	0.20	0.20	0.15	0.09	0.20	0.55	0.50	0.49	0.06	0.02	0.04	0.07	0.10	0.12	0.14
	144	0.240	2.4	0.54	0.64	0.72	0.64	0.43	0.18	0.20	0.20	0.15	0.09	0.20	0.55	0.50	0.49	0.06	0.02	0.04	0.07	0.10	0.12	0.14
	145	0.240	2.4	0.54	0.64	0.72	0.64	0.43	0.18	0.20	0.20	0.15	0.09	0.20	0.55	0.50	0.49	0.06	0.02	0.04	0.07	0.10	0.12	0.14
	146	0.240	2.4	0.54	0.64	0.72	0.64	0.43	0.18	0.20	0.20	0.15	0.09	0.20	0.55	0.50	0.49	0.06	0.02	0.04	0.07	0.10	0.12	0.14
	147	0.240	2.4	0.54	0.64	0.72	0.64	0.43	0.18	0.20	0.20	0.15	0.09	0.20	0.55	0.50	0.49	0.06	0.02	0.04	0.07	0.10	0.12	0.14
	148	0.240	2.4	0.54	0.64	0.72	0.64	0.43	0.18	0.20	0.20	0.15	0.09	0.20	0.55	0.50	0.49	0.06	0.02	0.04	0.07	0.10	0.12	0.14
	149	0.240	2.4	0.54	0.64	0.72	0.64	0.43	0.18	0.20	0.20	0.15	0.09	0.20	0.55	0.50	0.49	0.06	0.02	0.04	0.07	0.10	0.12	0.14
	Mean	0.240	2.4	0.54	0.64	0.72	0.64	0.43	0.18	0.20	0.20	0.15	0.09	0.20	0.55	0.50	0.49	0.06	0.02	0.04	0.07	0.10	0.12	0.14

M. GASTROCNEMIUS MEDIALIS.

Discussion

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TABLE E—(continued)
Details of Rabbits and Mice.

M. CRISTOFANETTI & M. BELLINI										M. PULINI & M. DE										
Star name		Wavelength (Å)		Wavelength (Å)	Data sources					Data sources										
					Length in Centimetre	Width at Base	Width at Apex	Depth at Apex	Depth at Base	Length in Centimetre	Width at Base	Width at Apex	Depth at Apex	Depth at Base						
106	101	2.09	1.17	1.77	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
121	101	1.17	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
47	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
41	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
114	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
115	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
116	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
117	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
118	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
119	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
120	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
121	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
122	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
123	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
124	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
125	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
126	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
127	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
128	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
129	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09
130	101	1.09	1.17	1.78	1.58	2.14	1.54	1.88	0.12	0.11	0.41	0.30	0.10	0.09	0.12	0.11	0.41	0.30	0.10	0.09

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